

Benefits from defence research?

All governments worry about the civil spin-off from defence research, but the British government has more reason than most to be alarmed.

DEFENCE research has always been the largest single identifiable component of the British government's spending on research. In the less self-conscious years before the Second World War, defence research was almost all of publicly supported research. For most of the period since, the proportion has comfortably exceeded a half, but in the past ten years it has been falling and is now, at £2,400 million a year, just under a half of all public spending on research — 49.2 per cent in 1986. Even so, that is three times what the government spends on basic research through the research council system; it is natural enough that the advisory committee called ACARD (for Advisory Committee on Applied Research and Development) should have singled out defence research for special study. The report, now published (by ACARD's successor ACOST, see page 87) is one of the most helpful studies of its kind since the Gibb-Zuckerman report of 1961.

The perpetual British dilemma about defence research is that, in the absence of major conflicts, the money spent on it is both an insurance of national security and an ostentatious waste. Indeed, in the early post-war years, it was worse than that — literally nothing less than a device for taking a small skilled labour force out of gainful pursuits. But as the numbers working in civil research and development have increased, there has emerged a continual lament that it seems not to be possible to conduct defence research in such a way that the benefits accrue to civil industry. Envious glances are cast across the Atlantic where, British legend has it, the US Department of Defense has discovered how to spend money on the procurement of new weapons systems in ways that improve the skills of civil industry. Why cannot the same be done in Britain is the lament, widely echoed elsewhere in Western Europe where the EUREKA programme of collaborative civil research is a French-inspired attempt to mirror the supposed civil benefits of the Strategic Defense Initiative.

Part of the answer is that much of defence research is misnamed. The report argues that a substantial part of British defence research is so directly related to particular weapons systems, or is such general work designed to help the armed services make up their minds what to buy next, that it is literally irrelevant to the needs of civil industry. The calculation is that the value of the defence research

that might be of civil relevance is at most £439 million a year, or just under a fifth of the total, not very different from the very similar proportion calculated for the United States. That is why the committee, like others previously treading the same path, has sought other explanations for the unsatisfactory transfer of skill from defence to civil industry.

Other explanations abound, but the ACOST report's most revealing argument (mostly tucked away in its Appendix G) is that Britain's defence ministry stands out from others in its refusal to "acknowledge any responsibility for helping safeguard and sustain the national technology base". This, of course, is a far cry from the United States, where the Pentagon has been a principal source of support for novel technology — integrated circuits, for example — on the grounds that the United States has a strategic interest in the technical skill of US industry. Other European states than Britain deliberately mate military and civil interests, with at least some success.

Yet the British government (whose instant comment on the ACOST report is unusually bound in with the text) says that the development of the British technology base must be largely left to "the operation of market forces in an environment of competition" and that, if the Ministry of Defence were to assume an "industrial sponsorship role or a responsibility for technologies unrelated to defence needs", the result would be "muddled roles and inefficiency". This puritan declaration, entirely consistent with the government's industrial policy, is administratively impeccable, but does it make practical sense?

For more than 40 years in Britain, the defence establishment has been too much of a law unto itself. During that period it has been responsible for a long list of research and development projects which, as events showed, were over-ambitious — from *Bluestreak* (the ballistic rocket), abandoned in the 1960s, to the development of an airborne early-warning radar, abandoned only last year in favour of Boeing's off-the-shelf product. The circumstances cry out for more awareness in Britain's defence ministry of the state of technology at the civilian companies from which it buys its weapons. The arrangements now developing in Europe, and particularly between Britain and France, may provide a better framework than at present, but placing orders for weapons that cannot be manufactured is a way of wasting money prolifically. As a



stopgap, there should be a procedure by which its research programme must be approved in advance by an appropriately qualified group of civilians who can be trusted to bring realism to its decisions.

But what companies? The ACOST report makes plain that too many of Britain's principal defence contractors are over-dependent on military work. Britain's largest company, the General Electric Company Ltd (no relation to GE of the United States) earned £1,300 million from the British ministry in 1986, some 22 per cent of its turnover in that year. (The chief defence contractors derive an even larger proportion of their research and development funds from the same source.) Remarkably, British defence procurement accounts for no less than 12 per cent of all British manufacturing output. If foreign arms sales were included, the total might approach 20 per cent. What Britain needs is a device for making defence contracting less attractive to those who are now engaged in it. One of the more useful suggestions in the report is that defence contractors should be encouraged in other directions by being required to pay for a greater proportion of defence research out of their own pockets — being recompensed by a greater rate of return whenever their projects succeed. But that would require a revolution which is unlikely to take place. □

Is TRON unfair?

Preparations for trade retaliation by the United States against Japan nonsense.

CAN it be a restraint of trade that the people to whom one wishes to export valuable goods happen to speak a different language? That, or something close to it, is one version of the question that has now arisen in the strained relationship on trade between the United States and Japan (see page 88). Among the disputed issues between the two countries listed last week by the new US Trade Representative, Mrs Carla Hicks, is a Japanese computer program, called TRON, intended to be used as the operating system for a line of personal computer systems not yet built. One attraction of TRON is that it is said to be better suited to the manipulation of the Chinese characters called *kanji*, the most distinctive features of written Japanese, than are the more familiar operating systems for personal computers, MS/DOS and UNIX. For this and, no doubt, other reasons, the Japanese education ministry has approved TRON alone for its programme to equip all Japanese schools with computers. Does that amount to a restraint of trade?

The simple answer is yes, but only in a way not touched by Clause 301 of last year's US Trade Act which, among other things, requires last month's listing of unfair trade practices and which will lead, at the will of the US Congress, to retaliation against governments not improving

their practice over a predetermined interval. TRON seems to have begun life not as a free-standing alternative to MS/DOS or UNIX, but as a scheme for the definition of standards for software and hardware interfaces that would make all kinds of computer systems more easily compatible with each other.

That the Ministry of Education should have decided to standardize on computers with TRON architecture is easily understood. A ministry that can rewrite school history books in ways that give profound offence in neighbouring China is easily capable of overlooking the implications for international trade of a decision on school computers. But unfortunately, there is nothing in the international rules of fair trade (GATT), to which both Japan and the United States subscribe, that outlaws chauvinism by public purchasers such as Japan's education ministry.

Now that TRON has become an international issue, the education ministry will no doubt back down. That may be the more palatable because of the doubts recently accumulated about the wisdom of hitching all of Japan's computer designs to a single untried standard. But the general principle remains that Japan's education ministry is as much within the GATT rules to specify the use of TRON in Japanese school computers as it would be for, say, the state government of California to specify that its school systems should standardize on MS/DOS or UNIX, perhaps on the grounds that software would then be more easily exchanged. The moral, for the US Congress, should be that TRON is not a substantial cause for complaint (others in Hicks's list may be), but that the urgent need is that all public purchasers play fair. Even within the European Economic Community, it is by no means certain that such a condition can be made to stick before 1992.

There is also a broader lesson to be learned from the gathering dispute between Japan and the United States on trade. Although Japan was blessed (by the late General Douglas MacArthur) with a constitution very much like that of the United States, the two countries are very different places. For all the daring of its engineers and the willingness of its salesmen to tramp the world in search of business, Japan remains socially and politically conservative. A Japanese government can no more easily snap its fingers at its rice farmers in the countryside for the sake of importing cereals at world market prices (a long-standing bone of contention) than a government in the United States could declare that Detroit no longer makes the best motor-cars. Yet the Japanese government has dutifully done its best, in the past few years, to accede to pleadings by the United States that it should buy more US goods. The agreement between the two governments two years ago on "reciprocity" in computer chips has not produced the intended effect because it was in itself an artificial restraint on trade. The best course for the US Congress, now, would be to put the federal deficit to rights, when the trade imbalance with the rest of the world would melt away. □

Disputed paper takes centre stage in Congress

■ Forensic data raise questions

■ Baltimore has day in court

Washington

LAST Thursday, 4 May, was a day many in the scientific community feared would have a chilling effect on research in the United States. But that was not the outcome.

Chairman John Dingell (Democrat, Michigan) and his colleagues and staff on the energy and commerce subcommittee on oversight and investigations spent nearly eight hours grilling witnesses about possible fraud or misconduct in the preparation of a paper that appeared in *Cell* in 1986.

At the end of the long day, Dingell proclaimed that he was "not satisfied with the answers we have been given" and promised that his subcommittee would continue to probe the matter. But the larger concern, that the investigation would lead to the formation of a 'fraud squad' to police scientific research, did not seem a likely outcome of the day's events.

The hearing had the ingredients of high drama. Waiting outside before it began was a body of illustrious scientists who had travelled to Washington from all over the United States to offer support for the *Cell* paper's authors, particularly for Nobel laureate David Baltimore, the paper's most famous author.

Inside, Secret Service forensics experts were standing by with giant displays showing retouched photographs and laboratory notebook pages out of chronological order. And finally there was the long-awaited meeting between Dingell and Baltimore, two men of great stature even in the exalted circles in which they walk.

Questions about the *Cell* paper (45, 247; 1986) were first raised in 1986 by Margot O'Toole, then a postdoctoral fellow in the laboratory of Thereza Imanishi-Kari, the paper's principal author. The paper deals with the expression of endogenous immunoglobulin genes in transgenic mice.

O'Toole's concerns were eventually taken up by Ned Feder and Walter Stewart, two National Institutes of Health (NIH) researchers known for their persistent investigations into misconduct in research. Baltimore declined Feder and Stewart's request for access to the data relating to the paper, and instead requested that NIH conduct their own inquiry. Ultimately NIH formed an investigatory panel chaired by Joseph Davie of Searle Research and Development.

But Feder and Stewart's case was taken up by Dingell's subcommittee, which in

April 1988 held hearings where O'Toole's charges were given a public airing. At the time, the subcommittee was criticized for holding a hearing without inviting testimony from the paper's authors, and since then there has been growing anticipation that eventually Baltimore would get "his day in court". He got it last week.

The hearing began with a long cross-examination of Davie and the other two members of the NIH panel — Hugh McDavitt of Stanford University and Ursula Storb of the University of Chicago — together with NIH director James Wyngaarden. Concern focused on whether the points raised in the panel's final report released in January this year constituted 'misconduct' or 'serious error'.

The panel had criticized the *Cell* paper's authors for overstating the specificity of the Bet-1 reagent used to distinguish between endogenous genes and the transgene, some errors that appear in two of the paper's tables and some inaccuracies in the text. But the panel did not find evidence of "fraud, misconduct, manipulation of data, or serious conceptual errors". After prolonged questioning, Davie admitted that some of the errors could be considered misconduct if the authors could be shown to have had an intent to deceive.

A letter of correction appeared in *Cell* last year (55, 541; 1988), and a second letter requested by the NIH panel is expected to appear shortly.

The NIH announced two weeks ago that they were reopening their investigation of the *Cell* paper (see *Nature* 339, 3; 4 May 1989). Wyngaarden explained that new allegations had been received from O'Toole that raised questions about the data used to prepare the paper prompting the case to be reopened. In particular, there was concern that data for one of the serial dilutions in figure 1 did not exist.

Then came the Secret Service testimony. The subcommittee last August asked the Secret Service to review the original data documents that were used to prepare the *Cell* paper. The seven-month investigation revealed that notebook pages that bore dates in 1984 were more probably prepared in 1986. The Secret Service also showed that an autoradiogram in figure 4 was a composite of more than one negative, and that the exposures had been adjusted so that some bands that appeared in the original negatives were not visible in the published figure.

So the stage was set for Baltimore's

appearance. In his opening statement, Baltimore blasted Feder and Stewart for their "vitriolic" campaign against him, and accused the subcommittee of "joining them in this relentless campaign". He called "a partial oral summary" of the Secret Service results presented to the paper's authors the previous week "a charade of helpfulness", and accused the committee of blindsiding the witnesses with new information on the day of the hearing. He further argued that the main contentions of the paper were correct, and that the work had been corroborated and extended in other laboratories.

A key point in the subsequent questioning focused on a letter from Baltimore to Herman Eisen at Tufts on 9 September 1986, in which he wrote that "The evidence that the Bet-1 antibody doesn't do as described in the paper is clear. Thereza's [Imanishi-Kari] statement that she knew it all the time is a remarkable admission of guilt."

At the hearing, Baltimore explained that the letter was written on the basis of a mistaken impression of a conversation between Eisen and Imanishi-Kari. The confusion was cleared up a few days later and he thought no more of the event.

For her part, Imanishi-Kari explained that the forensic evidence proved nothing more than the fact that she was not a "neat" person, and that the dates in the notebooks were occasionally out of sequence because of the way she kept her records. She further pointed out that her work was aimed at finding a cure for lupus, and that as someone who suffered from the disease herself and had a sister who died from it, it would be unthinkable for her to make up data that would in any way mislead scientists working in the field. Baltimore added that the sum of the Secret Service data showed merely that different scientists do things differently.

As for the autoradiogram in figure 4, Baltimore and David Weaver, the paper's first author, insisted that the contrast had to be adjusted so that more relevant data would not be obscured.

In the end, Dingell castigated Baltimore and his colleague for failing to be more forthright in their answers to the subcommittee's questions, but before he could bring down his gavel to close the hearing, Baltimore insisted on a final statement in which he expressed his outrage at the way he had been hounded by the subcommittee and its staff.

Despite the confrontation between Baltimore and Dingell, both congressmen and staff struck a more conciliatory tone. They insisted that their concern was more for the process of conducting investigations into allegations of misconduct, and that they were anxious to see research institutions and NIH do a better job, which NIH had shown they could do if prodded.

Joseph Palca

Still no certainty

Los Angeles

ANY expectation that a verdict on the reality of cold fusion would soon be delivered remained unfulfilled after the special session of the Electrochemical Society's meeting in Los Angeles last Monday. Martin Fleischmann and Stanley Pons retreated on some of their claims but firmly defended their measurement of the heat output of their electrolytic cells against recent charges of sloppy experimental technique. By the end of the evening, the University of Utah researchers and their critics were still at a standoff on most of the disputatious issues, but the audience was clearly unhappy at Pons and Fleischmann's reluctance to take up offers of help from other scientists to analyse parts of the experiment more closely.

In contrast to the previous week's meeting of the American Physical Society in Baltimore (see *Nature* 339, 4; 4 May 1989), where Pons and Fleischmann were convicted *in absentia*, the Los Angeles meeting was both more sympathetic and more hopeful. Nevertheless some naysayers, notably Nathan Lewis of the California Institute of Technology, were able to get on to the programme.

Responding to assertions by Lewis in Baltimore that their temperature measurement was flawed, Pons and Fleischmann showed a video recording of one of their cells in action, in which obvious and vigorous bubbling was adduced as the means by which a uniform temperature was maintained throughout the cell. Furthermore, a dye (phenolphthalein) introduced into the electrolyte was visibly well-mixed in about 20 seconds. But Lewis said later on that the calibration of the cell's energy output was still of unquantified accuracy. In Pons and Fleischmann's set-up, a relation between heat flow and temperature differential is obtained by the use of a resistive heater, through which a measured energy is passed before electrolysis is begun; what remains undemonstrated, according to Lewis, is that the calibration, performed when the electrolyte is still, is a useful measure of what happens when the cell is working and the electrolyte is seething with evolved gases.

But on the question of whether fusion by-products had been seen there was a retreat. Fleischmann said that criticisms of their gamma-ray line at 2.2 MeV, that it was too narrow and too weak to be the neutron-capture line they claimed, were ones that he too had worried about and expressed to Pons before publication, and that they were working on new measurements. And claims that a significant production of helium-4 had been detected (see *Nature* 338, 691; 27 April 1989) were premature.

Support for cold fusion came from

Robert Huggins of Stanford University, who repeated his claim that a heavy-water electrolytic cell ran consistently hotter than an identical light-water one, and from a number of speakers from Texas A&M University, who showed calorimetric data indicating that a palladium cathode in a heavy-water electrolyte produced excess heat, while platinum in heavy water and palladium in light water produced nothing out of the ordinary. Fleischmann declared that any suggestions that control experiments done with ordinary water were yielding excess heat comparable to that produced with heavy water were "total nonsense".

But Steven Jones of Brigham Young University emphasized the need to have detections of some fusion products; anomalous heat alone does not prove that nuclear processes are at work. It was mentioned repeatedly that helium is retained by palladium for up to 12 years, and that if heat was being generated at the rate claimed by Pons and Fleischmann, a post-mortem of an electrode would reveal high helium concentrations. Members of the audience offered to analyse a piece of one of the Utah electrodes, but Pons declined, saying that arrangements had already been made, with a laboratory he would not identify, to do similar analyses.

GLOBAL WARMING

Row in the Senate over altered testimony

Washington

AN ATTEMPT by US President George Bush's Office of Management and Budget (OMB) to modify congressional testimony on global warming caused outrage earlier this week at a Senate subcommittee hearing on "Possible climate surprises predicting greenhouse warming". James Hansen, head of the US National Aeronautics and Space Administration (NASA)'s Goddard Institute for Space Studies said that OMB altered his written testimony and made him appear to negate his own predictions of global climate changes by saying they were only "estimates" and not "reliable predictions".

The hearing came as administration officials attending the United Nations Intergovernmental Panel on Climate Change in Geneva were briefed to go slow and recommend more studies during discussions of international agreements to protect the atmosphere. The Bush administration reacted coldly to a Senate resolution calling on the United States to take the lead in setting up an international convention to slow global warming.

Senator Albert Gore, Jr (Democrat, Tennessee), who chaired the hearing, said the changes in Hansen's testimony had been made by "a nameless, faceless

At the end of the session, no useful resolution had been reached. Those who argued that cold fusion works also admitted that reproducibility was difficult. Robert Huggins claimed that the palladium had to be cast, not annealed, and that it had to be purged of hydrogen; U. Landau of Case Western Reserve University described some preliminary suggestions of excess heat from annealed electrodes. J. Bockris of Texas A&M University said that out of twenty palladium electrodes tested by three different collaborations at the University, only 5 or 6 produced heat. To explain these mystifying variations, Bockris argued that fusion occurs when large concentrations of deuterium build up around defects and dislocations in the palladium (see this week's issue, *Nature* 339, 105; 1989), but this made no impression on Nathan Lewis who said that the group at the California Institute of Technology had tried 50 electrodes, some cast, some annealed, and none of them worked.

No true discussion between boosters and critics occurred. At a later press conference, Fleischmann said it was difficult for him to respond to Lewis's criticisms because he had not seen all the data in a published paper, with all details made available. According to Pons, they hoped to publish a full paper describing the calorimetry behind his and Fleischmann's results later in the summer. **David Lindley**

bureaucrat" from a "kind of science Politburo". Preventing the best scientists from giving their best data to Congress "is a form of science fraud" and an "outrage of the first order of magnitude", said Gore. Committee member Senator Richard Bryan (Democrat, Nevada) added that Bush seemed to be fulfilling his campaign promise to "counter the greenhouse effect with the White House effect" by using the "whitewash effect" instead.

Hansen said that more than a year ago he chose to appear before Congress as a private citizen rather than accept changes to his testimony made by OMB. All formal testimony by government employees — including government scientists — must first be vetted by the administration.

At a press briefing, White House spokesman Marlin Fitzwater defended the Administration's action by saying "OMB's position was that it was a policy issue and not a scientific one". He conceded that the changes were made by an OMB employee at least "four levels down from the top", and that Hansen was in his rights to present his own conclusions during oral questioning, even if they differed from his approved testimony. When asked if he was worried about retributions for his candour, Hansen replied "I am now". **Carol Ezzell**

POLISH UNIVERSITIES

Compromise disappoints

London

POLAND'S academics, while delighted with the re-legalization of Solidarity, are disappointed with the compromise which is the outcome of their six weeks of negotiation with the government at the so-called round table, which brought together government and opposition interests.

The universities had been hoping that the 1985 Higher Education Act would be annulled, returning the regulation of Poland's universities to the framework of the earlier legislation inspired by the Solidarity period of 1980–81. But, instead, there is to be a new law from the autumn of 1990 restoring to universities a measure of control over financial, curricular and staffing decisions. Meanwhile, interim regulations will, from next September, bringing an end to some of the most resented restrictions.

Thus the Minister of National Education will no longer be able to veto the granting of doctorates on ideological grounds, and representatives of students and non-teaching staff will once again

participate in senate and departmental committees. Lecturers dismissed on ideological grounds have been promised reinstatement, although it is not clear how their seniority can be restored without the creation of further posts. And, starting immediately, permission to travel abroad on academic business is in the gift of university rectors; the consent of the Ministry of National Education will no longer be required.

Although Solidarity has been legalized, there is a delay in dealing with the pro-Solidarity Independent Students' Association (NZS). The authorities are still pressing for a formula to limit the militancy of NZS, whose disturbances at Kraków during March at one stage imperilled the whole concept of the round table.

NZS held its second national conference at Wrocław last month. A major split developed between 'radicals', who refuse to accept government constraints, and moderates who urge that, while the organization remains illegal, it has no means of making students' grievances known except

by demonstrations.

Among other things, NZS is concerned about the condition of the Warsaw University library, where underspending and the neglect of conservation for several decades have put at risk many of the collections of rare and historical manuscripts. NZS organized a demonstration on the issue in mid-April, but says that if it were given legal status, it could be more constructive, perhaps organizing fund-raising drives.

Meanwhile, there will be several distinguished academics among those standing for the forthcoming elections to the bicameral parliament. The candidates for the Senate include Dr Władysław Findeisen, the former rector of Warsaw University who was sacked in 1985 for having refused to discipline students attending the funeral of the murdered priest Father Jerzy Popiełuszko.

The candidates for the lower house (Sejm) cover almost all scientific and academic disciplines. There are four mathematicians, one physicist, one zootechnician, two geologists, an astronomer — as well as two students.

Vera Rich

SPACE ADMINISTRATION

West Germany founds space agency

Munich

THE West German cabinet agreed on 26 April to coordinate its space programme through a new private agency, to be known as DARA (Deutsche Agentur für Raumfahrtangelegenheiten GmbH). The agency will be overseen by a committee of cabinet members led by the Research and Technology Minister.

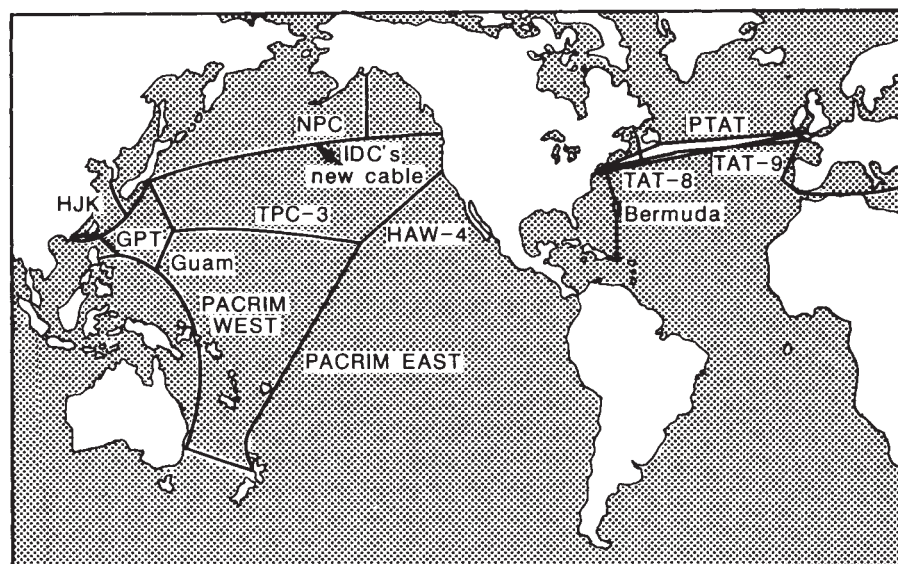
By organizing DARA as a private agency, the government hopes to gain flexibility in the planning and carrying out of space missions. The agency will be supported by contracts from various ministries for tasks such as remote sensing, telecommunications and research. The DARA budget will start at DM30 million and increase to DM55 million in the near future. The agency is expected to have 300 employees.

Establishing the agency took years despite an obvious need. A political struggle between the recently reorganized German Aerospace Research Establishment (DLR) and the Research and Technology Ministry accounted for some of the delay (see *Nature* 328, 6; 1987).

Two vital decisions remain for DARA. A director has to be nominated by a board of directors, which itself still has to be formed. And a site has to be found. Chancellor Helmut Kohl has decreed that the agency should be located in Bonn's immediate surroundings.

Steven Dickman

International telecommunications



A NEW Japanese telecommunications company established by Cable and Wireless of the United Kingdom began international services last week. The company, International Digital Communications (IDC), was the centre of a trade dispute with Britain and the United States two years ago when Japan at first refused to grant the foreign-backed company an operating licence (see *Nature* 326, 319; 1987). The costs of international telecommunications in Japan are expected to drop significantly as a result of the new competition.

IDC is initially offering leased data-transmission services at rates 20 per cent below those of Japan's international telecommunications giant Kokusai Denwa Denwa (KDD). Another new telecommunications company, International Telecom Japan, which is backed by a consortium of Japanese companies, also began a similar service last month. Ordinary telephone services will begin in October, according to a spokesman for IDC.

All three companies are at present using Intelsat satellites and a new trans-Pacific optical-fibre cable (TPC-3), which was completed last month. But IDC and Cable and Wireless will also lay their own trans-Pacific cable (NPC) which will come on line in December next year. IDC's new cable, which will link Japan to Alaska and Oregon via a submarine branching point off the US coast, will form part of a global optical-fibre network connecting Europe, the United States and Asia.

David Swinbanks

Destruction area disputed

São Paulo

DISPUTES about the true extent of rain-forest destruction in Brazil have triggered a minor war in the country's Institute for Space Research (INPE, Instituto de Pesquisas Espaciais).

Brazil's president, José Sarney, claimed on 6 April that INPE figures showed just 5.12 per cent of the area of the 'Legal Amazonia' had been destroyed since colonization began in 1500. But the true figure may actually be close to 12 per cent according to Vitor Celso de Carvalho, who heads the institute's department of research and applications of remote sensing.

The first estimate was certainly incorrect because it did not relate the deforested area — 251,429 square kilometres — to the total area of forest but to the whole of Amazonia (of which only about 75 per cent is forest). A recalculation taking this into account places deforestation at 6.8 per cent (see *Nature* 338, 531; 1989).

But that error is just the tip of the iceberg. A second edition of the report includes "ancient deforestation" data too. The area destroyed jumps to 343,975 square kilometres, or 9.3 per cent of the estimated 3.7 million square kilometres of forest. Adding to this the destruction in other kinds of Amazonian vegetation, including the savannah-like 'cerrado', the figure approaches 12 per cent.

Carvalho strongly criticizes the haste with which data was produced for the president's announcement. He says that the short time available to prepare the first report and the lack of reliable data — the team had to use older satellite images for some regions, instead of only 1988 material — "compromised" the work, causing serious damage to the institute's image and credibility.

INPE's first report describes deforestation "up to 1988", taken by Sarney in his speech to mean deforestation "since the discovery of Brazil", and "up to 1988". But the institute's director, Márcio Nogueira Barbosa, says that it meant "recent deforestation" only. The 92,546.43 square kilometres of ancient destruction that must now be added is an area three times that of Belgium.

Although Barbosa said earlier that the report was the "most complete" study ever of deforestation in Brazil, anomalies remain. An earlier work by the now-defunct Brazilian Institute for Forestry Development (IBDF, Instituto Brasileiro de Desenvolvimento Florestal) shows that 8,132 square kilometres of forests were destroyed in the state of Acre by 1987. INPE's reports say the figure is 5,509 square kilometres to 1988 — meaning that Acre has "gained" a forest area of 2,600 square kilometres. Both institutes used

Landsat data.

At the same time that the controversy was raging in INPE's headquarters at São José dos Campos, São Paulo State, Brazil's president was playing host to his colleagues from Amazonian countries in Manaus, in the state of Amazonas. The reunion of the presidents of the Treaty of Amazonian Cooperation ended last Sunday with tirades against foreign intervention in the region's affairs and complaints about external debt payments.

Those present — the presidents of Brazil, Ecuador, Guiana, Surinam, Peru, Venezuela, Colombia and the foreign minister of Bolivia, which is in the middle

ORAL CONTRACEPTIVES

Increased cancer risk found

London

THE most comprehensive British study so far of the oral contraceptive pill and the risk of developing breast cancer provides evidence for a substantial causal relationship between prolonged use of the pill and an increased risk of breast cancer in young women. The new study adds to the morass of contradictory evidence on whether or not a link exists, and although not conclusive it swings the balance further than before in favour of a link.

The case-control study involved 755 women diagnosed with breast cancer before the age of 36, and was carried out by researchers at the Imperial Cancer Research Fund, the Cancer Research Campaign and the University of Oxford. The results, published in *The Lancet* last week, show an increase in the risk of breast cancer of about 40 per cent after 4–8 years of pill use, and an increase of about 70 per cent after more than 8 years, although pills with lower doses of oestrogen seem to be associated with a lower risk, and progestogen-only pills seem to have a protective effect.

The risk of young women in Britain developing breast cancer is still low: about 1 in 500. But the new study suggests that one-fifth of the 650 cases a year can be ascribed to pill use. The principal researcher, Clair Chilvers, says women should now aim to use pills with the lowest dose for the shortest possible time.

The Committee on Safety of Medicines (CSM), the government advisory body, is taking a cautious attitude to the findings, pointing out that these risks would be expected to produce a detectable increase in the reported incidence of breast cancer in young women. The fact that no increase has been observed, and the lack of correlation with the risk estimates, cannot be ascribed to under-reporting, it says. But Professor Julian Peto, one of the research

of presidential elections — also said that the growing concern of developed countries for the environment should be translated into financial assistance to less well-off countries.

The lack of scientific knowledge of the region was well demonstrated last week when the Brazilian National Foundation of the Indian (FUNAI, Fundação Nacional do Índio) announced its first contact with a tribe of 130 Indians in a remote area in the north of the state of Pará, near the Surinam border. Brief contact with the tribe had been made seven years ago by a group of missionaries. In order to make the trip, the FUNAI team had to borrow a helicopter from Petrobras, the country's state-owned oil company.

Ricardo Bonalume Neto

team, says that registration is unreliable and cannot be used as evidence either way.

In a letter sent to general practitioners last week, the CSM recommends no change in prescribing oral contraceptives, seizing on the fact that the study is concerned mainly with the use of older oral contraceptives which contain higher doses of oestrogen than are the norm today, and that for ovarian and endometrial cancer the pill may be protective. But Chilvers says this attitude is "over-reassuring". She supports the response of the Family Planning Association and the National Association of Family Planning Doctors which both say that women should continue to use their present packet of pills but consult their doctor.

The US Food and Drug Administration reported in January that the evidence for a link between the pill and breast cancer was so conflicting and inconsistent that it was difficult to draw conclusions, and recommended no change in present pill use. A large US study providing evidence against a link has now been challenged by Peto. Carried out for the Cancer and Steroid Hormone Study Group and reported in 1985 in *The Lancet*, that study concluded that use of the pill had no effect on risk of breast cancer before the age of 45. After reanalysing the data, Peto comes to the opposite conclusion.

The new British study is now being followed up with a similar one involving women between the ages of 36 and 45 in an attempt to answer the vital question of whether the perceived risk is transient and diminishes at older ages. A study last year by the Royal College of General Practitioners suggested that this was the case, but the researchers say the risk may have been undetectable in previous studies, particularly if there is a latent period between exposure and risk.

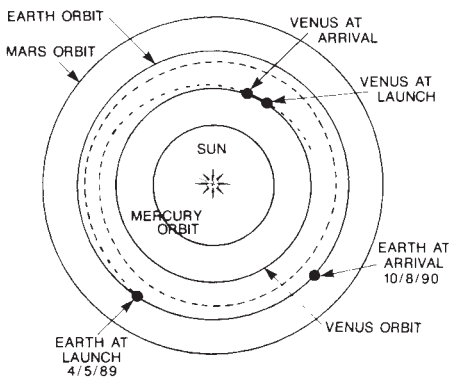
Christine McGourty

PLANETARY SCIENCE

Magellan's search for data

Washington

AFTER lengthy delays and one false start, the Magellan planetary probe was last week launched on its way to Venus aboard the space shuttle Atlantis. When it arrives at Venus late next summer, Magellan's radar remote-sensing instruments will peer through the clouds to provide a detailed surface map of the planet that most closely resembles Earth. But the avalanche of data it will relay to Earth during its eight-month mission is expected to double that generated by all previous planetary missions combined, and will stretch the current analysis capabilities of the US National Aeronautics and Space Administration (NASA).



Magellan is the first new planetary probe since the Pioneer spacecraft was sent to Venus in 1978 to provide a rough 60-mile-resolution map of the planet. Plans for a higher-resolution map of Venus were made later that year, but the launch date was postponed twice and the entire mission was cancelled once because of budget cuts and the greater emphasis on the shuttle programme.

The 1986 Challenger accident further delayed a second trip to Venus, as well as postponing plans for the Galileo exploration of Jupiter, the Mars Observer probe and the Ulysses mission to study the poles of the Sun. Magellan secured the fourth spot on the shuttle manifest once it resumed flying last year. The trip to Venus will take one year longer than if it had been launched in 1986, because the different alignment of the planets means it must first travel once around the Sun.

When it arrives in August 1990, the Magellan spacecraft will orbit Venus 1,852 times over a period of eight months to map 90 per cent of the venusian surface in 16-mile swathes. Magellan will map only one side of the planet during each of its three-hour elliptical orbits; when it moves to the other side, it will transmit the collected data to Earth. The resolution of Magellan's synthetic aperture radar is ten times that of the previous Pioneer probe, and should provide enough detail to work out whether or not the crust of Venus is

split into tectonic plates or is solid like that of the Moon.

Magellan is the first planetary exploration mission to fly aboard the shuttle and not on an unmanned rocket. When the shuttle was designed, NASA decided to place all space-science flights on the shuttle — a decision that was hotly debated once the shuttle was grounded after the Challenger accident. A second planetary mission, Galileo, is scheduled to be launched by the shuttle in October.

DEFENCE RESEARCH

UK advisers want civil links

London

BRITAIN'S Ministry of Defence (MOD) was accused last week of being "indifferent" to the long-term health of the country's technological base. After a two-year review of defence research, the government's Advisory Council on Science and Technology (ACOST) says that as large amounts of public money go to defence research, greater benefits should accrue in the civil sector than do at present. In 1986–87, £2,400 million was spent on defence research and development, but less than 20 per cent was aimed at technology with possible applications in the civil sector.

In striking contrast to the policies of most other countries, the British government does not positively encourage technology transfer between civil and military research, says the ACOST report. It recommends that the MOD should take greater account of the country's future technological capability, mainly by encouraging investment in a broader technology base for defence from which both defence and civil sectors may benefit.

The MOD should identify technologies in which it can influence the direction of civil-driven research efforts, participate more with industry in developing technologies with civil as well as military applications and award a greater number of research contracts to universities and research institutes with proven tech-

AIDS

Transfusion damages

Paris

FRENCH haemophiliacs who have contracted the AIDS virus as a result of transfusion with contaminated blood products (before these were systematically screened) will receive an award for damages. A settlement plan outlined by the government last week will give the 12,000 victims who have developed AIDS awards of between FF50,000 and FF150,000 (\$8,000–\$24,000).

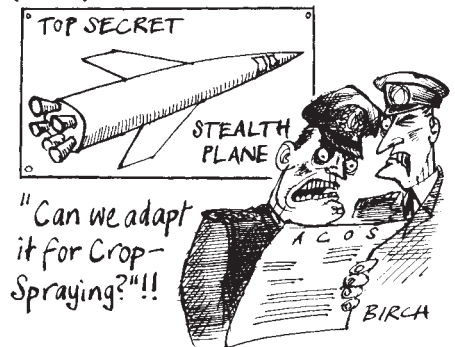
Peter Coles

While orbiting Venus, Magellan will generate billions of bits of data. To help integrate the mountain of telemetry, NASA has plans for a \$5 million-per-year Planetary Data System, which will involve a network of 10 computers located at Washington University in St Louis, the Massachusetts Institute of Technology, Brown University, the United States Geological Survey in Flagstaff, Arizona, and the Jet Propulsion Laboratory in Pasadena, California. But the sheer volume of the data will mean that much of it will remain unanalysed for several years.

Carol Ezzell

nology-transfer mechanisms.

The ACOST report also recommends restructuring of the MOD's own research establishments, which at present are "declining in vitality and viability". Because of recruitment problems, the quantity and quality of staff is now inadequate in some sectors even to maintain the MOD's capacity to act as an informed customer when procuring technology. This problem may be alleviated in part by formation of the new Defence



Research Agency which will absorb at least four of the establishments and will not be tied to rigid civil service pay scales (see *Nature* 338, 287; 1989).

Formation of the agency, which loosens the ties between the ministry and its research establishments, is a step in the right direction, says Sir Charles Reece, chairman of the group which produced the ACOST report. But he said the ministry should focus on fewer areas of technology and aim for higher standards. He would like to see independent technology centres which are national centres of excellence serving both defence and civil markets.

The Ministry of Defence is carrying out its own review of the role of its research establishments, but says the MOD should not have responsibility for technologies unrelated to defence research because that would lead to "muddled roles and inefficiency". It does agree, however, with the broad objective of maximizing cross-fertilization between defence and civil research.

Christine McGourty

Car phones fuel battle

Tokyo & Washington

IN line with tough new trade legislation, the United States last week released a 'hit list' of 54 Japanese high-technology products targeted for possible sanctions because of Japan's refusal to open fully its telecommunications markets. Additional sanctions may follow because of Japan's failure to import other US high technology products, including supercomputers, semiconductors and satellites.

Frustration in Congress over Japan's reluctance to buy US products continues to increase. The Omnibus Trade and Competitiveness Act of 1988 placed new requirements on the Office of the US Trade Representative to provide a clear picture of why US products were not selling better abroad. At the end of last month, the office released two reports that indicate rocky times for US-Japan trade relations.

The review of telecommunications agreements concludes that Japan has violated the MOSS (Market Oriented Sector Specific) agreements of the mid-1980s by limiting access of a car telephone system developed by the US company Motorola. Under US pressure, Japan's Ministry of Post and Telecommunications allowed the Motorola system into Japan, but only in the western part of the country. Eastern Japan, which includes Tokyo and Nagoya and comprises 70 per cent of the car-telephone market, is reserved for a competing system developed by the domestic telecommunications giant Nippon Telegraph and Telephone Corporation (NTT).

Furthermore, whereas the NTT system is allowed to 'roam' into western Japan using alternative radio frequencies, the ministry has refused to allot such frequencies to the Motorola system in the Nagoya-Tokyo area.

The annual report on trade barriers covers dozens of countries and a complete spectrum of trade from sales of US supercomputers in Japan to barriers to US exports of apples to Norway. But Japan takes up more pages than any other country and most of the trade problems with Japan involve high technology. The report will be used by the US trade office to designate 'priority countries' that will be subject to investigations that could lead to sanctions. The countries will be selected by the end of this month and Japan is again expected to be included.

Many US high-technology products that sell well elsewhere in the world have failed to penetrate the Japanese market, according to the report. The US optical-fibre cable industry, which the report says is among the world's most competitive, has managed to capture only 3 per cent of Japan's \$500 million-a-year market.

Similarly, sales of US telecommunica-

tions satellites have been meagre. A few satellites have been sold to private telecom companies that have tie-ups with US companies. But NTT, Japan's biggest telecommunications company, is obliged to buy more expensive Japanese satellites because the government, the major shareholder in NTT, wants to nurture the domestic satellite industry.

The United States is particularly dissatisfied with the failure of Japan's universities and government research institutes to buy US supercomputers, a technology in which the United States is the acknowledged world leader — although Japanese manufacturers are not far behind. A 1987 bilateral agreement was intended to simplify public procurement of US supercomputers. But only two machines have been sold, and those were bought under a special import promotion plan on a non-competitive basis.

Semiconductor trade is an arena in which the United States has been mauled by Japan. But the United States continues to lead the world in development of many types of advanced microchips and microprocessors. The report criticizes Japan for failing to increase access of US chips to the Japanese market as agreed under the 1986 US-Japan Semiconductor Agreement.

TRON (The Real Time Operating System Nucleus), a new 'open architecture' for computers invented by Ken

Sakamura of Tokyo University, is a newcomer to US-Japan trade disputes. Although no commercial TRON computer is yet available, a council set up by the Ministry of Education, Science and Culture (MESC) to recommend computers for high schools has laid down specifications that favour TRON operating systems. Furthermore, NTT announced in January that it will require TRON architecture for its next-generation digital communications network.

The US trade office's report on trade barriers expects that US computer operating systems (such as MS-DOS and UNIX) will be effectively excluded from MESC's lucrative \$7,000 million procurement of computers for high schools. Looking further to the future, there are US fears that Japanese manufacturers will 'design in' TRON microprocessors and 'design out' US parts, thereby further aggravating the semiconductor dispute.

Coincident with release of the two reports, plane-loads of Japanese government officials arrived in Washington to try to head off US retaliation. Japan's vice-minister of post and telecommunications, Yusai Okuyama, offered Motorola access to the whole of Japan for the next generation of digital car telephones and Hiroshi Mitsuzuka, minister of international trade and industry, promised to increase public sector procurement of US supercomputers. Further Japanese concessions are expected before the end of the month.

David Swinbanks & Joseph Palca

US OBSERVATORIES

University of Arizona construction agreed

Tucson

DESPITE demonstrations and a 350-page appeal from an environmental and conservation group, the US Forest Service has approved the University of Arizona management plan for a new observatory in southeastern Arizona. Last month, the Forest Service issued a special-use permit authorizing construction of three telescopes, support facilities and an access road on Mount Graham, a 10,700-foot peak that is also home to an endangered species of red squirrel.

Last November, Congress passed a bill authorizing the observatory (see *Nature* 335, 755; 1988) with the understanding that measures outlined in a US Fish and Wildlife Biological Opinion to protect the squirrel were followed. The Mount Graham Conservation Project and Earth First! Biodiversity Project submitted a 350-page appeal challenging that opinion and the Forest Service assessment of the observatory's environmental impact. The group had requested a stay on the special-use permit until the appeal was considered. According to the project coordinator, Jasper Carlton, the opinion is

inconsistent with the Endangered Species Act, and the university's management plan is inconsistent with the opinion.

However, the Forest Service refused that request. "Congress has not given us a whole lot of options", says Pat Jackson, appeals and litigation officer for the regional office of the Forest Service in Albuquerque, New Mexico. Now that federal law incorporates the observatory plan in the biological opinion, the Forest Service must carry it out. "We don't feel that anything inappropriate has been done." As part of the management plan, which was developed by the university and the Forest Service, biologists will begin a 10-year study of the squirrel and will monitor the squirrels close to the construction site. A squirrel refuge is being established, roads near important habitats will be closed and areas will be reforested. Three telescopes will be built initially and four more are planned, depending on the effects on the endangered squirrel.

Last month, protesters from Earth First! and the Sierra Club demonstrated at the university and at Mount Graham.

Elizabeth Pennisi

DNA FINGERPRINTING

Pitfalls come to light

Berkeley

THE application of DNA fingerprinting to forensic samples is gaining rapid acceptance in the US criminal court system. In less than two years, DNA data have been considered as evidence in more than 80 criminal rape and murder trials in 27 states, leading to at least 64 convictions or pleas of guilty by the defendants. But some biologists and legal experts have expressed concern that the evidence is not as infallible as judges and juries are being led to believe.

The power of the technique is not in dispute. When performed correctly, it can match DNA from an individual to that extracted from samples of blood, hair or semen left at the scene of a crime. The most commonly used method relies on restriction fragment length polymorphisms (RFLPs), regions of DNA that vary among individuals, producing fragments of different sizes when the DNA is cut with restriction enzymes and run on sizing gels. When probes for a large enough number of RFLPs are used, the likelihood of a match between two unrelated individuals may be less than 1 in 100 million.

Forensic DNA testing is at present carried out by three commercial US laboratories, and the Federal Bureau of Investigation (FBI).

Concern about the tests focuses on the potential for human error in sample treatment or data analysis, the interpretation of DNA patterns, the uniformity of criteria used to determine whether two samples match and the population studies on which the predicted likelihood of a mismatch is based.

In written testimony presented to Congress during hearings on DNA fingerprinting in March, law professor Barry Scheck, a member of the New York Governor's Commission on Forensic DNA Typing, writes that in many of the court cases there has been "little, if any, informed cross examination of private

DNA vendors, and few qualified expert witnesses testifying in opposition. The defence lawyers in these cases . . . have been overwhelmed."

That trend may be changing, as the defence in a murder case now under way in the Bronx has lined up an impressive string of biologists to testify to the potential weaknesses in the analysis of DNA extracted from a blood spot on the defendant's watch. The witnesses included human geneticists Eric Lander and David Page of the Whitehead Institute, Conrad Gilliam of Columbia University and Howard Cooke of the Medical Research Council in Edinburgh.

Among the issues raised in the case was the lack of adequate controls in the DNA analysis performed by Lifecodes Corporation, a New York company specializing in DNA fingerprinting. Lander challenged Lifecodes' decision to discount two non-matching bands as contaminants, even though no experiment had shown that the bands were not actual human DNA differences. In addition, Lander questioned the company's conclusion that the blood on the watch came from a female, based on a sex-determination test performed with a male-specific probe. The autoradiogram used to make the DNA fingerprint contained no positive control to show that the probe was functioning properly, and Lifecodes scientist Michael Baird testified that the autoradiogram could nevertheless be interpreted in the absence of a positive control.

Cooke, who developed two of the probes used by Lifecodes in the DNA analysis, questioned whether one could match samples with certainty by eye, because variants in the population often produce DNA fragments of similar sizes. In traditional DNA diagnostics used in prenatal screening, the parents' DNA provides at most four separate bands with which the child's DNA must be judged to match or not, Cooke says. But forensic use involves much more variability, and therefore demands well-defined standards for determining a match.

The strength of DNA fingerprinting data lies in the potentially low probability of a match occurring between two unrelated samples. But Lander warns of a potential error that could arise in the reporting of those probabilities, if standards for a match are not uniform. The stringency of the standards for what constitutes a match determines the number of identifiably distinct variants of a given RFLP that exist in the population, and that, in turn affects the probability that a match might occur at random.

On the basis of both Lifecodes' testimony in court and unpublished scientific papers introduced into evidence, Lander

testified that a less stringent matching rule was used to determine whether one forensic sample matched another than the rule used to determine the probability that such a match could occur at random. The result, according to Lander, could be an understatement by a factor of 20 of the actual frequency that a match might occur at random for a given RFLP. If this were to occur for each of the RFLPs in a case, he says, the total probability could be understated by a factor of 1,000 or more.

There is no set of guidelines to help individual testing laboratories to make decisions about matching criteria and other procedures, nor is there a standard proficiency testing or licensing process for laboratories that perform the DNA analysis.

An independently conducted blind trial of commercial laboratories was carried out in 1987 by the California Association of Crime Laboratory Directors. Although the laboratories knew they were under investigation, a worrying number of errors was revealed.

Fifty samples, including semen stains, blood stains and hair were sent to the three commercial US testing laboratories. Two of the laboratories mistakenly matched unrelated samples. Both laboratories say they have identified the source of the problem, in one case non-binding of DNA to a filter, and in the other the accidental mixing of two samples. While measures have been taken to avoid such mistakes in the future, the blind trial points out the possibility of human error.

John Huss, vice-president of Cellmark Diagnostics, a Maryland subsidiary of Imperial Chemical Industries, and the company that accidentally mixed two samples, says the error led the company to take on additional quality control measures. Operations are now observed by a second technician, he says, and the observations recorded in a company log book.

Nevertheless, as caseloads grow, pressure on the private companies is intense, and could lead to errors, warned Cooke. That pressure may eventually diminish, as state and local crime bureaus set up their own laboratories.

US Representative Don Edwards, who chaired the congressional hearing on DNA fingerprinting, has called for licensing and regular proficiency testing of private laboratories and state and local criminal laboratories that perform the tests. A National Academy of Sciences study that was likely to have provided recommendations for standards of data analysis was recently postponed last year when neither the FBI nor the National Institute of Justice could provide funding. A report on DNA fingerprinting by the Congressional Office of Technology Assessment is due out by the end of the year. Although it will make no recommendations, it may provide the information Congress needs to design regulatory legislation. **Marcia Barinaga**

RESIGNATION

Koop out early

Washington

US SURGEON General C. Everett Koop announced last week that he is resigning with effect from July. He indicated in February that he did not intend to serve out his term, which would have expired in November. Koop took an unexpectedly high profile position on AIDS and the need for education in AIDS prevention during a time when others in the Reagan administration had little to say about the disease. Koop also worked hard to reduce the incidence of smoking in the United States.

Joseph Palca

Showpiece reactor on ice

Munich

THE malaise that has afflicted West Germany's nuclear industry grew worse recently when Research and Technology Minister Heinz Riesenhuber refused to increase government investment in a showpiece nuclear reactor. The decision cast doubt on the ability of the reactor to operate economically, an important selling point for the West German companies that intend to market reactors of this type worldwide.

Following the apparent abandonment by West Germany of the nuclear fuel reprocessing plant at Wackersdorf (see *Nature* 33, 611; 1989), Riesenhuber's announcement reinforce the impression that the government is wavering in its support for nuclear power. Riesenhuber and other government officials have denied this interpretation.

The showpiece reactor is a prototype high-temperature, gas-cooled reactor (HTR) with a generating capacity of 300 MW. Located at Hamm-Uentrop in northwestern Germany, it was the first HTR of its size in the world. The West German government paid 80 per cent of the estimated DM4,000-million final cost of building the reactor. Maintaining the non-operative reactor costs DM18 million per month.

Potential HTR producers such as Siemens and Asea Brown Boveri (ABB) are seeking overseas markets to make up for stagnating demand for reactors in West Germany, Switzerland and other Western European countries. Siemens and ABB have explored the possibility of exporting reactors to the Soviet Union and China, but no plans have been made.

Proponents of this reactor type say that it is inherently safe and that the high temperatures produced at the core — up to 950 °C — can be used as a second source of energy, for example to liquefy coal. But ironically, concern about safety led to the initial shutdown of the West German HTR in October 1988. The reactor has not operated since then.

Riesenhuber said no to a request from the reactor operators, Hochttemperatur Kernkraft GmbH (HKG), to increase government investment in the operating costs of the plant from the current DM450 million to over DM1,000 million. Much of this increase would have been used to produce new fuel elements for the HTR. The current supply of fuel will last for only two to three more years.

The only question seemingly still open is whether the HTR will operate at all using the remaining fuel elements before being decommissioned as early as 1991. HKG has requested further operation; Riesenhuber and local authorities have rejected it.

Decommissioning costs could run as high as DM400 million if the site of the HTR is returned to the 'green field' it used to be, as the government has promised.

Other countries are confident that HTRs will comprise the next generation of nuclear reactors. Japan has built a test reactor and the United States has for years operated an HTR in Fort St Vrain, Colorado. The US Department of Energy is proposing building an HTR in Idaho to produce tritium for nuclear weapons.

The future of the partly built nuclear fuel reprocessing plant at Wackersdorf was still uncertain last week, although a number of factors point to the plant being shut down. Even the Bavarian government, which initially defended the project, has agreed in principle to a

shutdown. Politicians and utility officials met in Bonn last week to debate Wackersdorf's future, but reached no decision.

At the same time, West German Environment Minister Töpfer, who is also responsible for reactor safety, travelled to Paris to discuss the French offer to take over West German reprocessing and sell West German companies a share of the French reprocessing facility at La Hague.

The end of Wackersdorf could also cause significant cuts in the West German research budget. West Germany at present spends about DM120 million a year on improving reprocessing technology. Up to one-third of this could be affected by the decision on Wackersdorf. The other two-thirds are spent on quality control and radiation-emission control of nuclear fuel elements, areas that will continue to be important even if Wackersdorf is shut.

Steven Dickman

US NUCLEAR POWER

Disposal plan for old military reactors

Richland, Washington

THE fate of the nation's eight retired plutonium production reactors at the Hanford Military Site in Washington State became clearer recently when the US Department of Energy released an environmental impact statement outlining disposal options. The controversial impact statement has been awaited by many nearby residents and other environmentalists ever since the last of these reactors was shut down in 1971.

The Hanford site has produced a sizeable share of the weapons-grade plutonium in the United States. The military reactors now considered for decommissioning include the nation's first such reactor which produced the plutonium used in the atomic bomb dropped on Nagasaki during the Second World War. Apart from the eight reactors included in the report, only one more recent plant, N-reactor, exists at the Hanford site, and it too has been shut since 1987 because of safety concerns.

Energy Secretary James Watkins has ordered that the slightly newer N-reactor be kept indefinitely in 'cold standby' status. But Hanford officials announced that nuclear fuel had been removed from N-reactor, quelling speculation that plutonium production at the site might soon resume.

The decommissioning options outlined in the Energy Department's report range in estimated cost from \$41 million for no action to more than \$200 million in fixed 1986 dollars. These estimates make up only a tiny fraction of the department's estimated \$57,000 million needed to effect a complete clean-up at the Hanford site.

An odd twist to the decommissioning

process is that the oldest facility at Hanford has been designated a national historic mechanical engineering landmark by the American Society of Mechanical Engineers. The B-reactor went on-line in 1944 under the direction of Enrico Fermi, and discussions are now under way about maintaining the reactor as a tourist attraction.

Seth Shulman

Reactors in Wonderland

Boston

THE Shoreham and Seabrook nuclear power plants continue to lurch slowly but steadily toward operation. With positive proclamations from the Nuclear Regulatory Commission (NRC) in each reactor's case, licensing no longer seems to be a serious obstacle for either plant. At this point, only state and local opposition will prevent the plants from going on line. At the Seabrook, New Hampshire, plant, Massachusetts Attorney General James Shannon is challenging in court a low-power license NRC is expected to issue. In New York, Governor Mario Cuomo's plan to buy the Shoreham plant from the Long Island utility for a token sum of \$1, and then dismantle it, still technically holds the day. But this plan has been weakened by NRC's decision to issue a full-power license for the plant, and the strong pronouncements from many leaders in the energy field supporting Shoreham's operation.

James D. Watkins, US Secretary of Energy, recently called the Cuomo plan for Seabrook "one of the most foolish deals in the nation's history". Calling the dismantling plans "utterly irresponsible", Watkins added "one dollar for a \$5.6 billion testament to American technological know-how, only to dismantle it? Even Alice's Wonderland does not contain such anomalies."

Seth Shulman

A view of misconduct in science

Efraim Racker

In the United States, suspicion of fraud has become a cancer in research. To tackle the problem, real and perceived, scientists will have to become more practised at public relations.

IN THIS article I give a personal view of misconduct in science. It is based on one experience in my own laboratory and three others in different laboratories, and I shall consider four aspects of the problem: the scientists who were involved; the supervisors and collaborators; the university and funding agencies; and the government as represented by Congress.

The scientists who were involved

I have had direct or indirect contact with four young scientists who were accused of fraud. The first worked in the laboratory of Dr Carl Cori at Washington University in St Louis; the second in the laboratory of Dr David Green in Madison; and the third in the laboratory of Dr Melvin Simpson at Yale University and later with Dr Fritz Lipmann at Rockefeller University. (Cori, Simpson and Lipmann all published retractions. Green retracted the work with which he was concerned at a public meeting of the Federation of American Societies for Experimental Biology.) Finally there is Mark Spector, who worked in my laboratory, and most of this article will discuss his case.

What did these scientists have in common? They had an outstanding intellect, they were all informed in their research field and recognized the missing links that if solved would represent a breakthrough. They were skilled experimenters and joined laboratories that were in the forefront of a particular research field and that were headed by an investigator with a reputation of integrity. As far as I know, none of them ever admitted being guilty. These are trademarks of the 'professionals' and are not universal. 'Amateurs', who for example publish data on patients that do not exist, are usually more readily discovered by colleagues and students. The spiritual damage caused by scientific fraud is irreversible, and those involved are and should be reported and prosecuted irrespective of whether financial losses are involved.

One evening in 1972 (the year of Nixon's Watergate) I received a phone call at home from a man in Washington, DC, who told me he was writing a book on fraud in science. I forgot his name and I don't know whether the book was ever published. He told me he knew that I was familiar with several scientists who had been accused of fraud, and that he had found out about a sin of my youth — an interest in psychiatry. He challenged me

to give him a psychological analysis of these men. I briefly outlined to him the common traits mentioned above. Because these three men were very smart, I pointed out, they must have known that in view of the excitement caused by their claims the work would be repeated promptly and if there was fraud it would be quickly detected. I concluded, therefore, that they were mentally unbalanced and subconsciously wanted to be caught. The man from Washington responded, "You mean, like Richard Nixon?"

In my opinion scientific fraud, perpetrated by what I call the 'professional', springs from an unbalanced mind. Perhaps as with most professional criminals they are emotionally and mentally ill, often seeking self-destruction. But I must stress, before being misunderstood, that such a view should have no bearing on our verdicts of guilt and punishment. In most cases psychiatry has no place in the courtroom unless it becomes more scientific than it is today. This view is not generally accepted and is incompatible with criminal prosecution according to current laws. Scientists who commit fraud are rarely psychotic and can and should be prosecuted.

Mark Spector, as I knew him, was a brilliant young man. He wrote poetry, painted and was a superb experimenter. A young scientist who watched him perform experiments at the National Institutes of Health commented that it was like watching Beethoven play the piano (I hope that the NIH scientist had a better knowledge of biochemistry than of musical history). Spector's lectures were spellbinders, and as a graduate student he received lecture invitations from many places, including NIH. He was also an excellent teacher and one of my other graduate students is still grateful for what Mark had taught him. Outside pressures can drive investigators to fraud, but it was not a factor in the four cases I knew. Spector worked long hours and late into the night. I told him several times to slow down, assuring him that with his skill and teaching talents he would get a faculty position at a very good university. Whatever pressure there was came from within.

When Mark came to my laboratory we were working on the role of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ in glycolysis of Ehrlich ascites tumour cells. I also had several postdoctoral fellows working on protein kinases in mammalian and plant cells. Based on experiments he claimed to have

done, Spector proposed that a cascade of several protein tyrosine kinases was responsible for the phosphorylation of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ and its inefficiency of operation in Ehrlich ascites tumour cells, a clever concept, compatible with our ideas on the mechanism of pump inefficiency.

Spector generated data by substituting radioactive iodine, which is widely used to label proteins, for radioactive ATP. He showed me the protocols and autoradiograms day by day. I suggested experiments; as usual, some of them worked, others didn't. He was always downcast when they didn't. He was invited by Dr Edward Scolnick at NIH to demonstrate some of his experiments, the first of which was a complete failure. He called me in desperation, suggesting that the enzymes might not have survived the trip. He told me where he stored them, and asked me to ship them by Federal Express. With the enzymes I sent him, the experiments worked. Needless to say, he must have had the iodinating reagents with him all the time. Dr Scolnick told me much later that the samples Spector left behind contained iodine; Mark was an artist of deception.

It was sheer luck that we discovered what he had done. We collaborated with Dr Volker Vogt, then an assistant professor at Cornell, who on examination of one of Mark's gels accidentally covered it with a glass plate and noted that the monitor still registered counts. He realized that the glass should have blocked the radiation emerging from radioactive phosphate but not those from iodine, and reported the observation to me.

Another way of deception that scientists should be aware of is the simple method of spiking. Spector claimed that he had discovered a new ATPase activity in a cytoskeleton preparation from Ehrlich ascites tumour cells. It was exactly what I wanted to hear and I myself started to analyse and purify the enzyme. Because Mark collected the cells for his other work, he just turned the triton-insoluble fraction over to me to keep me busy. After three weeks of intensive bench work I became concerned, because the properties of the enzyme clearly resembled a well-known viral ATPase that we had studied. Sure enough, with electron microscopy we detected a few virus particles in the preparation. Soon thereafter Mark's preparations became weakly active or inactive. I thought that some of our mice may have been infected

by a virus and dropped the project. After the iodine incident, I realized that he could have spiked the cytoskeleton preparation with samples of the AMV virus which I had stored in my deep freeze. Mark knew where everything was kept.

One of Mark's claims was that he had isolated a new protein kinase activator with properties similar to a transforming growth factor described by Dr George Todaro. We received a sample of this factor from Dr Todaro. Mark was excited; it worked in his assay. Dr Todaro came to our laboratory and repeated the experiments with the enzymes he was given. He returned to NIH with a sample of Mark's purified activator and, at the next meeting of the Federation of American Societies for Experimental Biology, reported that Mark's factor was active as a growth factor. I suspect that what he tested was Mark's activator spiked with the growth factor Dr Todaro had generously given to us. There was a similar spiking incident with an antibody he had sent to Dr Ray Erikson. Dr Edwin Krebs was excited by our findings and sent his collaborator Dr Linda Pike to work with Mark. Needless to say, the data were spectacular and we were ready to publish when the iodine surfaced. Dr Pike, herself a talented experimenter, was duly impressed by Mark's skilful experimentation.

I am reporting these incidents here in some detail and for the first time, mainly to make a point that I shall re-emphasize later — it is difficult to catch a 'professional'. I shall describe in the next section how we responded.

The supervisors and collaborators

Supervision. Careful supervision of technicians, students and postdoctoral fellows is essential — not because of fear of fraud, but because it is part of our teaching responsibility to detect errors, inappropriate controls and so on. It has been a long-standing practice in my laboratory that important discoveries are repeated either by myself or by other colleagues. The reasons for that practice are that it detects experimental errors, it enhances confidence and it greatly helps in the write-up to ensure duplication in other laboratories. Some of my own experiments are duplicated by others in my laboratory. Many of Mark's experiments were repeated by me with enzymes given to me by him, and his experiments on the activator were duplicated by Dr Todaro. One possible explanation is that the enzymes he gave us were spiked — for example with the cAMP-dependent protein kinase, the activator preparation with cAMP — but I have no proof of this.

I would like to describe in some detail one particular incident that convinced me at the time that Mark's discoveries were genuine. At an early stage he showed me data that documented the

phosphorylation of the β -subunit of an $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ isolated from Ehrlich ascites tumour cells by an endogenous protein kinase. It was exactly what I had hoped for and, I, thought it was too good to be true. I asked a postdoctoral fellow in my laboratory to repeat the experiments and to explore other $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations that I stored in my deep freeze. Among them was an $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from *Torpedo californica*, which has a β -subunit with a different electrophoretic mobility to that of the mouse enzyme. A postdoctoral fellow reported to me that she could not repeat Spector's experiments. Mark was called in, quizzed her and quickly discovered that, by not freezing his enzymes in liquid nitrogen, she had failed to follow his instructions exactly. The experiment was repeated with fresh solutions and it was a remarkable success. All ATPases were phosphorylated on the β -subunit, including the *Torpedo* preparation with its more rapidly moving β -subunit. In later years I have shown this autoradiogram to many visiting experts asking how it could have been faked. Nobody could understand it. It was only when I wrote this article that I thought of an explanation that is staggering — during the night Mark could have found her protocol as well as the ATPase preparations in the deep freeze, labelled them with iodine, and placed them on an acrylamide gel which he substituted for hers.

Response to suspected fraud. The day after Vogt informed me that he had discovered iodine in Mark's samples we confronted him, demanding an explanation. He was quite calm, denied any wrongdoing and claimed that somebody else must have put the iodine into his samples. I felt it was important not to announce a premature verdict, and therefore gave him three weeks to prepare fresh enzymes which he was to turn over to me for assay with my own reagents. When he failed to come through, I notified the graduate school to withhold his doctoral degree for which all requirements had been fulfilled. I withdrew two papers in press and wrote a letter to inform NIH. I also sent a letter of retraction to *Science* (213, 1313; 1981) in which a review paper of our work had previously been published. Because some of Mark's experiments had been repeated by myself and others, several postdoctoral associates later attempted to determine what could be salvaged. We published several papers that were based on Mark's findings but there were enough differences to discredit all of his claims.

University and funding agencies

How should these institutions handle the prosecution? Some of our faculty members felt that Mark should be taken to court and punished. I decided not to recommend this route to Cornell University, for the following reasons. Once

again, Mark never admitted that he had committed fraud, and I felt that the evidence of one experiment at Cornell and one at NIH in which radioactive iodine was found instead of radioactive phosphorus could well not have convinced a jury or a judge. Mark would have made an eloquent and persuasive witness. He could not have denied the presence of iodine but could have denied that he had added it. Had we lost the case, Mark would have demanded his PhD degree and would have received it. As it was, he refused my request to resign voluntarily and threatened a lawsuit. I talked to Mark's mother, a reasonable lady with a great deal of insight, who finally persuaded him to withdraw without further action.

This brings me back to prosecution. Irrespective of causes, society must punish fraud. Although I don't know what eventually happened to Mark, his scientific career was destroyed and he gained no money to flee to South America. Such punishment is severe. In fact, I received quite a few letters accusing me of dealing too harshly with him and failing to give this brilliant young man a second chance. As far as I was concerned this brilliant young man was ill and there was no cure for his illness. Because I felt this way, I was never angry with Mark but was not willing to be lenient either. (The young scientist from Cori's laboratory was given a second chance and became a professor at the School of Public Health of Johns Hopkins University. He published several sensational papers that were never verified, and died at a young age.)

It seems obvious that each case must be handled individually. When the evidence can stand up in court, conviction and gaol sentences are in order. There may be mitigating circumstances, such as undue pressure and confession, which may warrant less severe measures — loss of reputation and a job is in itself severe punishment.

The government and Congress

I think that scientists should take the stand that fraud, when established, is a matter for the courts and we should show that we are serious about it. The obligation to meet legal standards as in other cases of fraud would discourage pursuit of trivial cases, that could be settled within the university or by NIH. It is sometimes impossible to distinguish between honest mistakes and fraud. We must try to educate the public and members of Congress that there is little to gain and a great deal to lose by involving Congress in scientific fraud. But Congress rightly insists that universities and funding agencies must forcefully investigate cases where fraud is suspected. We have to convince members of Congress that the operation of science is similar to that of self-defrosting refrig-

erators, that the occasional ice of fraud is melted away by the heat of progress.

Audits will have little effect on fraud and the expense is not justified. The argument of Dr Drummond Rennie, a deputy editor of the *Journal of the American Medical Association*, that we must persuade Congress not to get involved in audits, is valid, but I disagree on how to do this. He recommends that we scientists should undertake the task, but this would be a grave mistake. At best audits will provide us with numbers that show that fraud is relatively rare, but I doubt that our politicians will accept such a verdict. Audits may discover misconduct in clinical trials that would probably come to light anyhow. But they will not uncover the 'professionals'. Let anyone look at Mark Spector's autoradiogram and at the protocols of the experiments with spiked enzyme preparation and try to discover fraud.

The threat of audits will not scare those intent on fraud; it will only increase their sophistication in keeping notebooks, and they will use symbolic gloves that will prevent the detection of the fingerprints of their deceit. Audits may pick up discrepancies, bad controls and inappropriate authorships, but it will be very difficult to differentiate between fraud, poor judgement and errors in interpretation. I think historians could find such errors in the papers of our greatest scientists. Let them study the history of glycolysis and oxidative phosphorylation — can we call the mistakes or errors of judgement made by Louis Pasteur, Otto Warburg or Peter Mitchell scientific misconduct?

Inappropriate authorship is a common and unacceptable practice that can be reduced without audits. Editors of journals can specify their requirements and demand a list of what each author has contributed, signed by all authors. Painful, yes, but better than audits. I am concerned that if scientists start to audit data of their colleagues, the next step may be audit of experiments. All this will be humiliating, expensive, ineffective and will not significantly change the overall picture of misconduct. If Congress insists, let it conduct audits by its own staff and discover what audits are worth.

We have to educate the public, the press and members of Congress that if there is any human endeavour in which crime doesn't pay, it is in science. We have to



Efraim Racker — a self-portrait.

convince them by documentation that science has made enormous progress in the past few decades and that the few cases of fraud did not slow it down, but that government interference and their lack of enthusiasm for science will. We have friends in Congress; we must ask them to help us. Congress has already done its job of persuading scientists and administrators to be more vigilant and to pay more attention to cases of alleged fraud. We have a crisis on our hands, but should not overreact.

Our task is to convince members of Congress that science is too fragile to be used as a political football. Most scientists of today can take the heat, but what about the next generation? Our students are already dismayed by the prospect of fighting for grants and university positions, and are turning to other and more lucrative

professions. Let us collect data and carry out interviews with students that will document this point. If young scientists are also threatened by government investigations of unAmerican and unscientific conduct, they will simply stay away from a scientific career.

To conclude, I have a few recommendations. We should present to Congress a brief yet precise plan of what we can do, and we need a science lobby that will prepare and present the plan. I don't know all that such a manifesto should include, but I have some suggestions. It should define a role for the universities and the departmental chairmen, to keep an eye out for irregularities as well as for undue pressures on students and faculty members. It should include a role for editors in dealing with improper authorships and for setting higher standards for the reviewing processes. Faulty citation of references is a form of scientific misconduct that is easily criticized but often innocent. Reviewers should be requested to take an active role in pointing out such omissions. Editors who do a sloppy and erroneous job of reviewing are also guilty of scientific misconduct and should be eliminated from editorial boards. Just as members of Congress, editors and reviewers of manuscripts and of grant applications should be accountable to a committee on ethics. We should plan to educate practising scientists on how to supervise better the work of students, not as a device to prevent fraud but because that is a responsibility of any teacher.

We should also convince the press that they need to help us in informing the public of what science is all about. I wish we could persuade Congress to think of curbing inaccurate and sensational journalistic versions of the truth; there must be freedom of the press but without the freedom to distort and invent. Members of Congress, I trust, are well aware of this problem. All this will require time and patience, as well as vigilance — remember how many decades it took to convince even a fraction of the public of the simple fact that smoking is hazardous to health? Education is a slow process and imaginative ideas to accelerate it are badly needed. □

Efraim Racker is Albert Einstein Professor of Biochemistry, Cornell University, Ithaca, New York 14853, USA.

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Use of growth hormones

SIR—So far as I know, there are no regulations as yet that “prohibit the use of bovine growth hormones in the fattening of cattle” (*Nature* 338, 281; 1989). Unless there is recent new legislation, of which I am unaware, your well-motivated leading article is ambiguous. I suspect that all your references to “bovine growth hormone” should be replaced by “anabolic steroids”, a totally different group of hormones. This suspicion is based on my knowledge of the European regulations on the use of “growth hormone”, which I have been informed is as follows:

(1) Council Directive 81/602/EEC prohibits the use in the Community of steroids (having androgenic, oestrogenic, gestagenic or thyrostatic activity) in food-producing animals and was implemented on 1 January 1988. The United Kingdom introduced the legislation on 1 January 1987 because the steroid products licensed at that time had recommended withholding times of one year.

(2) The Hapart report (*Hormones and the BST hormone in the dairy and meat industry*) was debated by the European Parliament on 5 July 1988. This called on the European Commission to conduct a wide-ranging inquiry into various implications of the introduction of bovine somatotropin (BST) into the Community. Notably, an amendment calling for a ban on BST in the Community was voted on separately and rejected by the parliament.

(3) On 16 September 1988, the European Parliament debated and passed a resolution on the use “of hormones in meat production”. This was concerned almost entirely with the use of steroids (referred to simply as “hormones”) in livestock fattening. However, one of the 13 resolutions approved by the parliament calls for “support for a world-wide ban on the use of hormones and other substances to improve productivity in livestock production and support for a world-wide ban on the production, marketing and use of hormone produced through genetic engineering aids for fattening and substances used to increase productivity in animal production as part of the current round of GATT negotiations”. Of a total of 518 European MPs, only 114 (22 per cent) voted. A resolution by the European Parliament is only an expression of opinion. European law is set by the Council of Ministers, who have only enacted the original steroid ban legislation.

A further recent development which has presumably prompted your article is the publication on 21 March 1989 of a report by the European Parliament’s committee of inquiry into “the problem of quality in the meat sector”. This will go to the parliament’s plenary session on 10 April. The committee comprised 15

MEPs and was chaired by Carlos Pimenta (Portugal). This report supports the existing steroid ban and calls for tightening of enforcing procedures for consumer safety reasons (against the best scientific advice). The committee also stated that it is “opposed to the registration of new genetically engineered growth hormones and recommends the setting-up of a permanent scientific programme to look into the questions raised by the ever-increasing use of high technology in the production of food”. This report was supported by nine of the 15 members. Five members dissented and felt strongly enough to produce their own minority report which supported the scientific evidence that the five licensed (steroid) hormones were “indisputably safe” and called for the commission to reconsider the ban.

R. B. HEAP

*Institute of Animal Physiology and Genetics Research,
Babraham Hall,
Cambridge CB2 4AT, UK*

SIR—I believe most scientists involved in assessing the safety, quality and efficacy of medicinal products or the potential application of new biotechnological techniques in farm animal production will applaud the reference in your leading article “More wicked ways with hormones” to an “intellectual millstone” for the EEC if the European Parliament accepts the recommendations of its committee of inquiry into the problem of quality in the meat sector (European Parliament PE 130073/A/Fin, 21 March 1989) and advises the EEC to continue the 1988 hormone ban.

Unfortunately, your article contains an error concerning the current situation in that the 1988 directive (88/146/EEC) bans the use of anabolic-type hormonal substances (including progesterone, testosterone, oestradiol-17 β , trenbolone and zeranol, previously licensed in the United Kingdom under the Medicines Act) but does not include ‘growth hormone’ preparations, that is, the somatotrophins. The β -agonists and recombinant growth hormone preparations are under consideration in some EEC countries but have not, as far as I am aware, been evaluated by the relevant EEC committees. However, in a closely argued minority report (CS/CCG/RAP/406) supported by a group of British MEPs and Professor Raftery of Ireland, which recommends the removal of the hormone ban, they cite that Commission staff confirmed that (1) they had no evidence of harm to animal or human, and (2) the ban was introduced for a mixture of political and commercial reasons, not to protect the consumer. It is to be hoped that both reports will be

examined carefully by MEPs, thus preventing a recurrence of the 1985 vote, when only 127 MEPs out of some 450 voted, and few (about 45) attended the debate.

However, there is also a disturbing new draft report to the European Parliament by the Committee on the Environment, Public Health and Consumer Protection (Doc B2 434/87 — rapporteur: Mr Ken Collins) which, in para 13, “recommends, as part of the current round of GATT negotiations, a world-wide ban on the use of hormones and other substances used to improve productivity in livestock production and support for a world-wide ban on the production, marketing and use of hormones produced through genetic engineering, aids for fattening and substances used to increase productivity in animal production”.

The current trade difficulties between the EEC and the United States, the increased incidence of use of previously banned dangerous anabolic agents, and problems with monitoring and policing the current hormone ban all stem from a previous 1985 report which led to the imposition of the current hormone ban. There is indeed, a bleak future for the development of human and veterinary drugs if the European Parliament similarly accepts this proposal, not least because research and development of both are inextricably interlinked.

G. E. LAMMING

*University of Nottingham,
Department of Physiology and
Environmental Science,
Sutton Bonington,
Loughborough LE12 5RD, UK*

Earthquake risk

SIR—The disastrous earthquake in Armenia (*Nature* 337, 107; 1989) drew attention to the study of the seismic risk of nuclear power plants. In the Soviet Union, Mikhail Gorbachev proposed an international examination of some sites.

As a seismologist, I took part in seismic risk estimation for nuclear power plants in Hungary (Paksh, 1987) and Czechoslovakia (Bogunichich, 1988). The results of the research are not classified secret, but they have not been published anywhere. Publishing rights are reserved by the governments of the respective countries.

I would like to draw attention to this problem. I believe that publication of our results, international examination of seismic risk at both sites and subsequent reinforcement of these plants (closing them if necessary) should happen as soon as possible.

M. SALGANIK

*Institute of Physics of the Earth,
Laboratory of Strong Ground Motion
Prediction,
Moscow 123810, USSR*

Halley's comet is quite young

Three years after the most recent apparition of Halley's Comet, estimates of the time for which the comet has been circulating in something like its present orbit seem to be converging on a few tens of thousands of years.

THOSE who prefer their astronomy to be spiced with poetic imagery are naturally, and perhaps rightly, inclined to regard comets as wanderers in the Solar System. The justification is the now-conventional view of where comets come from: out there, beyond Pluto, is a collection of objects consisting of interstellar dust cemented together with water-ice and other kinds of ice. Gravitational perturbations of this Oort cloud, perhaps by passing stars, then deflect some members of the cloud downwards to the region of Neptune and Uranus whence, perhaps by the influence of Saturn and Jupiter, some of them may be deflected into the inner Solar System, to appear occasionally, but regularly, in the visible night sky.

The poetic image is apt. The chance that a particular member of the Oort cloud will be captured in this way is only small, while an object captured into the outer Solar System is more likely to be expelled from the Solar System altogether than to finish up in an orbit that carries it near enough to the Sun to be a spectacular apparition every now and again. In this accepted view of the appearance of comets, the existence of the Oort cloud itself is not a firm reality, but is inferred, essentially from calculations of the chance that such an object will be captured into the inner Solar System and from the number of known comets. In the circumstances, many people would be happier if there were more objective evidence for the reality of the Oort cloud.

None of that implies that inner Solar System comets are other than transient. By definition, objects such as these which are noticeable in the sky make spectacular sights because of the material they shed during their relatively close passage of the Sun, which implies that they cannot last forever. Indeed, the rate at which comets such as Halley lose material near perihelion is so great that they cannot have been in their present orbits for very long, either.

So it is a reasonable question to ask how long Halley's comet has been in something like its present orbit. It is not, of course, a new question, but now a group of three Canadian astronomers have produced what may be a more reliable estimate of the age so far of Comet Halley (Jones, J., McIntosh, B.A. & Hawkes, R.L. *Mon. Not. R. astr. Soc.* **238**, 179; 1989).

Jones and McIntosh, from the University of Western Ontario and the National

Research Council of Canada at Ottawa respectively, have a long-standing interest in the properties of the stream of dust ejected from Halley's comet which are recognizable from the surface of the Earth as the Orionid and η -Aquarid meteor streams (visible in October and the first week of May respectively).

The two meteor showers consist literally of material lost from Halley's comet which is now spread out in a region ranged about the comet's present orbit, and which the Earth intercepts regularly twice a year. If only it were possible accurately to estimate the total mass of the meteor shower, it would be possible to estimate the amount of mass the comet loses at each apparition much more accurately than can be told even from the accurate direct measurements made in 1986.

This is what Jones, McIntosh and Hawkes have been able to do on the basis of an earlier intricate calculation of the likely form of the meteor stream from Halley (*Mon. Not. R. astr. Soc.* **235**, 673; 1988). Against naive expectation, the dust particles ejected from the comet are not arranged in some kind of cylinder running the length of the comet's orbit, but are in the form of a thick ribbon-like structure whose position is determined not merely by all past positions of the orbit of Halley's comet, but also the influence of radiation pressure on the dust the comet has released in clumps during its infrequent perihelion passages.

This calculation confirmed previous guesses by the tedious if effective way of simulating the fate of particles of different sizes by the effects of gravitation and radiation on 500 test particles in each computer run. The upshot of the modelling is that the ejected particles occupy a ribbon-like region of space which is 4 per cent of an astronomical unit thick and ten times as wide. (An astronomical unit is the diameter of the Earth's orbit, or roughly 300 million km.)

That the seasons for the Orionid and η -Aquarid meteor showers bear little relation to the times of the year at which the orbit of Halley's comet now crosses the Ecliptic is simply a measure of the relatively high rate at which Halley's orbit is changing under the influence of Jupiter on the comet at aphelion — the most pronounced change of the orbit is a steady rotation about the major axis in a clockwise direction seen from perihelion.

How much meteor mass is locked up in

this ribbon? The only objective answer to this question must be the use of radar observations to estimate the amount of ionization left in the atmosphere by meteors in the shower. Among other things, information of that kind makes it possible to calculate the size-distribution of the dust particles in the meteor streams. The upshot of the calculation, in which the greatest uncertainties must stem from the numerical calculations of the dimensions of the ribbon, amounts to 1.3×10^{13} kg.

That is an arresting number in itself, for this amount of dust is a substantial fraction of the likely mass of Halley's comet proper. Although the mass was not accurately determined at the 1986 apparition, Jones *et al.* estimate from the accurately measured volume of the nucleus of the comet, and from its probable density, that the mass left intact must be only one order of magnitude greater than the dust scattered into the ribbon-shaped alley in which it now travels since Halley's comet was first captured into the inner Solar System. But because most of the comet's mass must consist of ice, not dust, there can hardly be more than two or three times as much dust to come as the quantity already released. Whatever the future for Halley's comet, it cannot be much longer than the time already spent within the orbit of Jupiter.

And how long is that? Jones *et al.* must necessarily start with some assumptions about the way in which material is lost from the nucleus of the comet at each perihelion passage. They take it that the amount of solar energy absorbed is proportional to the declining surface area of the nucleus and make assumptions about the proportion of this energy used to sublimate cementing ice (which may be expected to diminish with each succeeding passage). Surprisingly, they find that their estimate of the lifetime (so far) of Halley's comet is relatively insensitive to assumptions about the proportion of the comet that consists of ice.

Their conclusion is that the time Halley's comet has spent in the Inner Solar System is a mere 23,000 years, perhaps enough for fewer than 300 revolutions of the orbit. This result agrees well with another calculation that there must have been a close encounter between the comet and Jupiter something like 20,000 years ago. Not often do such long chains of inference come out so well.

John Maddox

The envelope thickens

Roger Chevalier

ALTHOUGH the recent appearance¹ of a putative pulsar at the heart of SN1987A brought this closest supernova back into the public eye, for astronomers the evolution of the expanding envelope of debris in the remnant has been of persistent interest in the past 2 years. Data reported by Teegarden *et al.*² on page 122 of this issue confirm that this shell of ejecta is more complex than anticipated. And Felten and Dwek³, on page 123, discuss the evidence for an unanticipated, pre-existing dust cloud behind the supernova. Furthermore, issues raised in 1987 by the brief appearance of a 'mystery spot' some distance from the supernova, have recently become further confused by several conflicting observations of knots of light.

The exponential decline in intensity of the light from SN1987A beginning in June 1987 gave strong evidence that the source of radiative energy in the envelope is the radioactive decay of ⁵⁶Co. Direct evidence came with the detection of the γ -ray lines given off by the decay process. As described by Teegarden *et al.* in this issue², it is now possible to measure the profile of the 1,238-keV line, which gives information on the spatial distribution of the ⁵⁶Co in the uniformly expanding supernova gas. Surprisingly the linewidth is large (full width half maximum, 4,000 km s⁻¹) and the line profile does not show the effects arising from opacity, although the line intensity is half that expected for the amount of ⁵⁶Co inferred from the light curve. A mean blueshift of the line was anticipated because emission from the receding (red-shifted) part of the supernova is likely to undergo electron scattering out of the spectral line on its way through the supernova. The lack of the shift implies that the smooth density in the γ -ray emitting region is lower than predicted.

At about the same time as the γ -ray observations by Teegarden *et al.*² (1 May 1988), accurate line profiles of Ar II and Ni II (singly ionized argon and nickel) infrared transitions were obtained by Witteborn *et al.*⁴ with the Kuiper airborne Observatory. Although these ions were not generated in the explosion (as the ⁵⁶Co was; ⁵⁶Ni would have decayed away by this time), the line profiles of Ar II and Ni II are similar and are probably representative of the heavy-element ejecta. The profiles show a redshift that can be attributed to scattering by free electrons in the expanding supernova. At optical and infrared wavelengths, the primary wavelength shift comes from the relative motions of parts of the supernova and the photons remain close to the line centre. The line profile of Ar II can be modelled by a constant-emissivity ion layer at velocities of 500 –

1,800 km s⁻¹ enveloped by an electron layer⁴. The ion velocity range is lower than that indicated by the γ -ray line, but may be consistent within the errors. Analysis of line profiles is crucial to understanding the mixing of elements in the supernova, for which there is no theoretical model.

Whereas the earlier spectra showed lines with redshifted peaks, from August to October 1988 optical lines developed an asymmetry to the blue⁵. This requires a source of continuous absorption; dust formation in the supernova is an excellent candidate⁵. The absorption is expected to reduce the optical luminosity, perhaps confirmed by the quickening decline in light intensity at 0.35–5 μ m during the period 27 July 1988 – 4 November 1988 (ref. 6). The optical luminosity had been falling below that predicted from the radioactive energy input. The test of the dust theory is that the absorbed energy is re-radiated in the infrared; the supernova's 10- μ m flux did increase from 2 June to 23 September 1988. The infrared and optical luminosity were comparable during this time. If supernova dust is the infrared source, the infrared luminosity beyond 5 μ m must be added to the luminosity at lower wavelengths to obtain a bolometric (total) luminosity. The flattening of the optical light curve at the beginning of November 1988, commonly attributed to an additional energy source such as a neutron star, long-lived radioactivity or an echo, may be due to a halt in the increasing absorption of optical light.

A difficulty with the dust model is that Roche *et al.*⁷ find the 10- μ m emission to be extended. Because the supernova is unresolved (pointlike), the emission would be from surrounding dust that has been heated by light from the supernova. This model requires that the dust cloud be placed directly behind the supernova and it is surprising that the silicate feature does not appear in the infrared spectrum because the surrounding gas has a high oxygen-to-carbon ratio, favouring silicate to graphite dust. Also, as discussed by Felten and Dwek in this issue³, an optical scattered-light echo would be expected from the surrounding dust. Crotts and Kunkel⁸ have set a limit on extended emission that is lower than predicted for standard dust models.

Although the detection of an infrared echo from circumstellar dust is controversial, there is now strong evidence for scattered light echoes. Echoes in the form of both a ring of emission^{8,9} and weak diffuse emission¹⁰ have been reported. The echo ring is consistent with a dust shell at about 5 parsecs from the supernova. A possible origin for the shell is interaction

of wind from the progenitor star during a red supergiant phase with the surrounding medium. The diffuse emission can be attributed to the undisturbed wind.

In addition to the diffuse emission, Heathcote *et al.*¹⁰ observed two knots of emission associated with the supernova, adding to a list of observations which has the unexplained 'mystery spot' at its head. This was observed in April 1987 by Nisensen *et al.*¹¹ using 'speckle' imaging techniques, but subsequently vanished. Karovska *et al.*¹², also using speckle techniques, detected a knot of emission at position angle from north (p.a.) 200°, 0.8" from the supernova. This position would be consistent with the position of the mystery spot were it moving radially from the supernova with a uniform velocity at least a third of the speed of light, *c*, beginning at the time of the explosion.

But the knots identified directly by Heathcote *et al.*¹⁰ on 7 March this year are at p.a. 45° and 270°. Furthermore, their observations, in the standard visible and near-visible detection bands, are inconsistent with there being a source of the type reported by Karovska *et al.* (seen in a 350-Å band centred on 5,510 Å) at p.a. 200°. Similarly, visible-band observations taken by Crotts and Kunkel¹³ during December 1988 conflict with the contemporaneous observations of Karovska *et al.*

On the other hand, Allen *et al.*¹⁴ reported in February 1989 the detection of narrow (less than 300 km s⁻¹) He I line emission (at 10,830 Å) from a knot at the same position as the Karovska *et al.* knot. This suggests the presence of a line feature in the Karovska *et al.* band which makes a smaller contribution to the broader visible band of the other observers. But such a strong narrow line close to 5,500 Å in the spectrum has not been reported. Narrow line emission is probably an echo effect from early supernova light and uniform echo expansion at *c*/3 requires a radial feature from the progenitor star at an angle of 37° to the line of sight behind the supernova. Resolution of the observational controversy is eagerly awaited. □

Roger Chevalier is in the Department of Astronomy, University of Virginia, Charlottesville, Virginia 22903, USA.

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Regulating the cell cycle

Geoffrey North

ACTIVELY dividing eukaryotic cells proceed through an orderly sequence of events termed the cell cycle, which in both mitotic and meiotic varieties culminates in the 'M-phase' of nuclear division. Following the discovery that homologues of a fission yeast cell-cycle control gene, *cdc2*, encode components of the biochemically defined inducer of M-phase termed MPF (maturation promoting factor)¹⁻³, attention is now focused on understanding what determines the timing of MPF activation at a particular stage in the cell cycle. Results presented at a recent meeting* implicate several proteins, in particular the cyclins, which are characterized by the intriguing way that their concentrations rise and fall in phase with the cell cycle, as key regulators of MPF activation.

The way that cyclins accumulate steadily during interphase only to be destroyed at M-phase suggested that they could explain the known requirement for new protein synthesis for the cell cycle to proceed from interphase to M-phase⁴. Furthermore, if the concentration of cyclins in a cell is a limiting factor in cell-cycle progression, then the rate of cyclin synthesis could provide a timing mechanism for at least part of the cell cycle.

Cyclins were discovered as major products of maternal messenger RNA in marine invertebrate eggs, and the first indication that they really are involved in cell-cycle control came when it was shown that cyclin mRNAs can induce maturation of frog oocytes⁵. The case became stronger with the finding that a mitotic-control gene of fission yeast, *cdc13*, encodes a protein with a sequence clearly similar to that of the invertebrate cyclins⁶.

M-phase entry

The cloning of complementary DNAs encoding two closely related cyclins of the frog *Xenopus* (T. Hunt, University of Cambridge) has enabled experiments to be carried out that demonstrate that cyclin synthesis is a prerequisite of entry into M-phase in a vertebrate system⁷. Hunt and colleagues have used their cyclin cDNAs to destroy the endogenous cyclin mRNAs in a *Xenopus* egg extract, and show that as a result the extract is blocked in the 'G2-like' state normally preceding M-phase.

Cyclin synthesis is thus necessary for entry into M-phase, but does it explain the protein synthesis requirement in full? Evidence that it does has been obtained in an elegant series of experiments reported at the meeting by Andrew Murray (University of California, San Francisco)^{8,9}. The experiments described by Murray

also used a cell-free system derived from *Xenopus* eggs, one that undergoes multiple cell cycles *in vitro*. Murray and colleagues have been able to destroy all the endogenous mRNA in such a system and show that cyclin mRNAs are sufficient to restore the ability of the extracts to cycle. Furthermore, by adding varying amounts of cyclin mRNA it has been found that in this system the rate of cyclin synthesis affects the length of the cell cycle: as the amount of added cyclin mRNA is increased, allowing a higher rate of cyclin synthesis, the cell cycle is shortened.

The implication is that a rate-limiting step for at least part of the cell cycle is the requirement for cyclin concentration to accumulate above some threshold level. Note, however, that protein-synthesis inhibitors cannot block entry into mitosis at any time during interphase — they can do this only when present during an early phase of the cell cycle, which implies that after the cyclin concentration has passed the threshold level a further slow step or steps is required before MPF is activated and M-phase induced. Furthermore, increased transcription of the fission-yeast cyclin gene *cdc13* does not lead to a shortening of the cell cycle, suggesting that in this system at least cyclin synthesis is not rate-limiting for cell-cycle progression.

Murray and colleagues have shown further that if a mutant, non-degradable form of cyclin is added to their system then M-phase is entered, but cannot be left. This provides strong evidence that the cyclin degradation that normally follows MPF activation has to occur for the cell cycle to continue out of M-phase.

How do cyclins regulate the activity of MPF? At present this is unclear, but the most likely mechanism involves interaction with the active component of MPF that is encoded by the *cdc2* homologue, p34 (so-called because of its molecular mass of roughly 34,000). Jim Maller (University of Colorado School of Medicine, Denver) reported that the 45K protein associated with p34 of *Xenopus* MPF is a cyclin, probably a breakdown product of the 56K *Xenopus* cyclin generated during purification of MPF. Draetta and colleagues have reported that complexes between p34 and cyclins can be isolated from clams, and argue that cyclins are essential components of active MPF¹⁰. Others, however, find that p34 can be active in the absence of cyclins (M. Doree, CNRS, Montpellier and C. Hutchison, University of Sussex); and Murray reported that if a *Xenopus* system is prepared with an endogenous inhibitor of MPF removed, after MPF has been

activated by cyclin addition the cyclin can be removed and the MPF remains active, suggesting that cyclins may have a catalytic effect on MPF activation.

Whether cyclins are essential stoichiometric components of MPF or catalysts of MPF activation, it is likely that post-translational modification of p34 is important in regulating its activity. It is known that p34 can be phosphorylated on several serine, threonine and tyrosine residues, and these modifications are likely to affect its activity. Paul Nurse (University of Oxford) reported that the p34 homologue in a human cell line is phosphorylated on serine at a stage roughly corresponding to the time during interphase at which DNA starts to be replicated (late G1-phase) and on threonine and tyrosine later in interphase, near the time that DNA replication is completed (late S-phase/early G2-phase). Nurse suggests that the serine phosphorylation is a prerequisite of MPF activity and the threonine/tyrosine phosphorylation is an inactivating step that has to be undone before M-phase can be entered.

Phosphorylation

Support for the view that MPF is activated by dephosphorylation comes from results presented by John Newport (University of California, La Jolla) and Maller¹¹. Both Newport and Maller find that dephosphorylation of p34 in *Xenopus* correlates with entry into M-phase. Newport has studied tyrosine phosphorylation of p34 in a cell-free system in which the endogenous MPF can be activated by addition of exogenous active MPF. The removal of phosphate from tyrosine groups of endogenous MPF follows addition of exogenous MPF, and is prevented by p13, an inhibitor of M-phase and homologue of the fission-yeast gene *Suc1*. Overexpression of *Suc1* in yeast delays M-phase and the gene is also required for exit from M-phase, presumably because it must be present for p34 to be phosphorylated and thus inactivated.

Much remains to be done to clarify how cyclins and p34 interact in the generation of active MPF, and also how they interact with other known regulators of MPF. Two genes have been identified in fission yeast that determine the timing of mitosis: *Wee1* is an inhibitor of mitosis, named by the small size of *wee1* mutant cells that enter mitosis prematurely; and *cdc25* opposes the action of *Wee1* and is an activator of mitosis, regulating the timing of p34 protein-kinase activation (Nurse). It is notable that *Wee1* encodes a protein kinase, suggesting it is responsible for an inactivating phosphorylation of p34. Little is known of the activities of the *cdc25* product, but it is intriguing that, like cyclins, *cdc25* protein builds up in fission-yeast cells during interphase, declining at M-phase (P. Russell, University of

*BSCB/BSDB meeting on the cell cycle, St Andrews University, 4-6 April 1989.

Washington, Seattle).

Functional homologues of the fission yeast *cdc25* gene have now been identified in other species. Mutations of the fruitfly *Drosophila String* gene cause cell cycle arrest during G2-phase of the fourteenth embryonic cell cycle, the first that is dependent on zygotic gene expression. Edgar and O'Farrell¹² have cloned the *String* gene, and find that the protein encoded by it is homologous to the fission yeast *cdc25* product. Expression of *String* in *Drosophila* embryos anticipates cell division, and it seems that *String* RNA cycles in a somewhat similar fashion to cyclin protein: high levels of *String* RNA correlate with the initiation of M-phase, and after M-phase the level of *String* RNA declines. The *String* gene will functionally complement fission-yeast *cdc25* mutations (Russell), strengthening the case that the two genes are functional homologues.

Russell and colleagues have also identified a budding-yeast gene that will complement *cdc25* mutations, which they call *MIH1* (ref. 13). Mutations of *MIH1* are not lethal, but they do delay entry into mitosis. Another property of *MIH1* is revealed by experiments in which *Wee1* is overexpressed in budding yeast. The budding yeast cell cycle differs from that of fission yeast in several respects — most notably it has not been clear that there is an important control point of entry into M-phase in the short G2-phase of the budding-yeast cell cycle. When *Wee1* is overexpressed in budding-yeast cells, it causes arrest of the cell cycle in G2, and cells that also have mutation in their *MIH1* gene are more sensitive to this effect. This strengthens the case that *MIH1* is a homologue of *cdc25*, presumably opposing the action of a budding-yeast homologue of *Wee1* that has not yet been discovered.

Although many aspects of MPF activation are poorly understood, still less is known of how MPF actually induces M-phase. One target of MPF is suggested by observations reported by M. Bornens (CNRS, Gif sur Yvette) that in HeLa cells at M-phase p34 is associated with centrosomes. Centrosomes are poorly understood structures that nucleate the formation of the mitotic spindle, and it is possible that p34 regulates this activity by phosphorylating centrosomal proteins.

Progress is being made in determining specific substrates of the protein-kinase activity of MPF. Maller and colleagues have shown that pp60^{src}, the product of the cellular homologue of the viral oncogene *v-src*, is a substrate of MPF kinase¹⁴. The pp60^{src} is phosphorylated *in vitro* by MPF at precisely the same serine and threonine residues at which it is phosphorylated *in vivo* in NIH 3T3 cells at M-phase, modifications associated with increased tyrosine kinase activity. Maller suggests that pp60^{src} mediates some of the pleiotropic effects of MPF, particularly those involving the cytoskeleton.

Contrary to a recent report¹⁵, Maller and colleagues do not find that pp60^{src} phosphorylates p34; indeed the significance of the observation that pp60^{src} can phosphorylate p34 on tyrosine is perhaps less clear following the evidence presented at the meeting that MPF is inhibited, rather than activated, by tyrosine phosphorylation. Nevertheless, the possibility remains that p34 and pp60^{src} are mutual substrates and some kind of feedback process is at work involving the kinase activities of these two enzymes. □

Geoffrey North is an assistant editor of Nature.

SOLID-STATE PHYSICS

Mass anomaly in superconductor

Alastair I. M. Rae

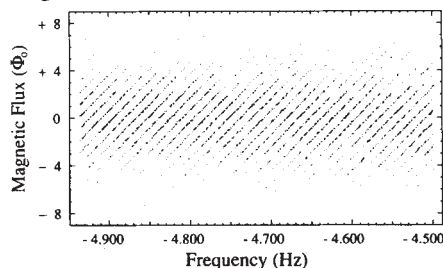
IN an article in *Physical Review Letters* (62 845–848; 1989) J. Tate, B. Cabrera, S. B. Felch and J. T. Anderson report a precise determination of the mass of the 'Cooper' pairs of electrons in superconducting niobium. This they achieved by measuring the magnetic field inside a ring of niobium while it was being rotated about its symmetry axis. Their result is 84 parts per million greater than twice the free-electron mass (m_e), whereas a theoretical calculation based on the theory of relativity predicts a value 8 parts per million less than $2m_e$. The physics behind the calculations and the reasons for the discrepancy raise some interesting issues.

It is generally known that the flux inside a thick ring of superconductor is quantized in units of the flux quantum (Planck's constant divided by twice the electron charge), but it is less widely realized that this quantization breaks down when the superconducting ring is rotating. This follows from the fact that the phase of the superconducting wavefunction is related to the generalized momentum of the charge-carrying Cooper pairs (which depends, in part, on the magnetic vector potential). When the ring is at rest, the charge carriers in the body of the superconductor where no current flows are at rest and their generalized momentum is therefore proportional to the magnetic vector potential in the superconductor; flux quantization follows from the requirement that the phase be single valued so that the line integral of the generalized momentum around the loop must be a whole number times 2π .

However, when the ring is rotating a further kinetic term equal to the product of the mass and velocity of the Cooper pair must be added to the momentum (see figure). The single-valuedness of the phase now implies the presence of an additional magnetic flux whose magnitude can be readily shown to be $(2m'/e^*)\omega A$ where e^*

and m' are the charge and mass of the Cooper pair, A is the area enclosed by the ring and ω is its angular speed. Clearly an experiment of this type provides a method of measuring the ratio m'/e^* .

A few such experiments have been performed over the past decade or so and they have all shown that (given that e^* is twice the free-electron mass from measurements of the flux quantum) m' is within 1 per cent of $2m_e$. What makes the new experiment different is the extreme care that has been taken so that a value for m' is obtained that is accurate to within a few parts per million (p.p.m.). The ring used was a thin-film band of niobium 20 μm wide and 40 nm thick deposited on a fused quartz rotor; the diameter of the ring was about 5 cm and this value was



SQUID output as a function of rotation rate over a 440-mHz interval. (From Tate *et al.*) measured interferometrically to an accuracy of 5 p.p.m.. The rotor was suspended on a helium-gas bearing at a temperature of around 6 K and rotated at speeds up to 5 Hz which were measured to seven-digit accuracy by reflecting light from a frosted pattern on the rotor top. The field inside the ring was measured using a superconducting flux transformer coupled to a 'SQUID' magnetometer.

Cabrera and colleagues found that their results were strongly affected by the presence of electrostatic charge on the surface of the rotor, but could monitor this by repeating their experiment with the ring heated above its superconducting transition temperature. Their final result

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is an average of over 600 measurements and yields a value for m' which is 84 ± 5 p.p.m. greater than $2m_e$.

At first sight, it might seem surprising that the value of m' is anywhere near $2m_e$. After all, surely the Cooper pairs are composed of electrons in the conduction band of niobium whose effective masses are very different from the free-electron mass. The idea of effective mass, however, is used to approximate the effects of the interaction between the electron and the lattice when the electron is moving *relative to the lattice*. In the present experiment, the charge carriers and the lattice are moving together so the effective-mass concept is irrelevant. It is generally agreed that the only corrections that apply in this case are those arising from considerations of relativity (mass-energy equivalence). The binding of the electron in the metal certainly means that the total mass of the electron plus the lattice is reduced by an amount corresponding to the binding energy — that is, the work function (ionization energy) in the case of the electrons at the Fermi surface (a characteristic energy in solid-state physics) which compose the Cooper pairs. Naively at least, we would expect this mass defect to be localized on the light electron rather than the heavy lattice, but what mass is actually being measured in the present experiment is a subtle and difficult question.

VISION

A shrimp's kaleidoscopic world

J.K. Bowmaker

BECAUSE light is not coloured, an animal's perception of colour is governed by the organization of its visual system: the number of spectrally distinct classes of photoreceptor within the eye; the spectral range they span; and the neural organization used to analyse the receptor information. Our own trichromatic colour vision is based on only three classes of photoreceptor, although many animals have systems based on four and probably even five channels^{1,2}. But is there a limit? Cronin and Marshall on page 137 of this issue³ show that the eyes of a lowly crustacean, the mantis shrimp, are remarkable in having as many as ten spectral classes of photoreceptor. This is by far the highest number reported in any species, either invertebrate or vertebrate.

The unique compound eyes of mantis shrimps are divided into three sections with an equatorial band of six parallel rows of ommatidia. Each ommatidium is built up of eight rhabdomeres, the cells that contain visual pigments. The central ommatidial band is itself subdivided in that the ventral rows 5 and 6 appear to be designed to detect the plane of polariza-

The theoretical figure quoted in this paper is 8 p.p.m. less than $2m_e$ and this is, indeed, equal to the work function in niobium which is 3.99 eV. However, the authors obtain this result by a more complicated argument in which a positive contribution of 180 p.p.m. from the kinetic energy of the electrons near the Fermi surface is cancelled by a contribution to the magnetic vector potential from the rotating charge density in the metal. The theoretical literature in this area is far from conclusive. In 1982, R. M. Brady (*J. Low Temp. Phys.* **49**, 1–17; 1982) found that the correction is the same as the work function, but in the same year Cabrera and co-workers (*Phys. Rev.* **B25**, 6644–6654; 1982) predicted an excess of 150 p.p.m. — about twice that actually measured. Apparently, a further paper giving more details of the theoretical calculations referred to in the present paper is to be published (B. Cabrera & M.E. Peskin, *Phys. Rev. B*; in the press). We shall have to wait for this and other theoretical work and also perhaps experiments on other superconductors before we can be sure whether the disagreement between theory and experiment has fundamental significance or is due to the details of the particular physical system being studied. □

Alastair I. M. Rae is in the Department of Physics, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK.

mostly orange and yellow, whereas in row 3 they become more exotic and varied between species and can be red, blue or purple (ref. 4 and see the discussion in News and Views by Hardie⁵). The colours of the filters result from concentrations of carotenoids and are reminiscent of the oil droplets found in the retinal cones of birds and reptiles⁶. In the ommatidial rows 2 and 3, after passing through the cornea and rhabdomere 8 (whose visual function is not known), light is filtered first on entering the first tier of rhabdomeres and again before entering the second tier.

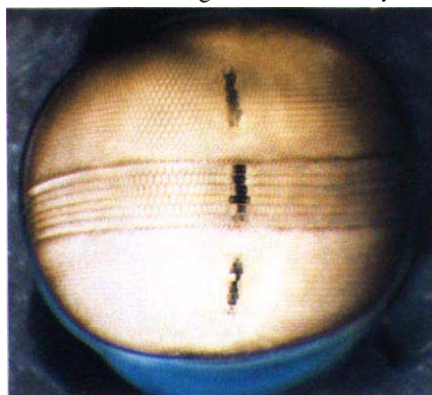
Cronin and Marshall have investigated microspectrophotometrically the visual pigments contained in the rhabdomeres of the different tiers. Each of the eight regions appears to have its own distinct visual pigment with peak absorbances ranging from 400 to 540 nm. Thus each ommatidial row has a pair of visual pigments, rows 1 and 4 with shorter-wave pigments (400–450 nm) and rows 2 and 3 with longer-wave pigments (500–540 nm). This is logical, because the filters in rows 2 and 3 have short-wave cutoffs and will pass only the longer wavelengths through to the underlying visual pigments. Similarly, the proximal visual pigment in all four rows is the longer-wave sensitive of each pair. The net result of the sequential filtering by both visual pigments and carotenoids is a set of four pairs of photoreceptors with narrow-band spectral sensitivities covering a range from about 400 to 640 nm.

Mantis shrimps thus have perhaps one of the most complex sets of photoreceptors in the animal kingdom — but what does this mean the shrimp can see? How colourful is its world? It has been suggested⁷ that because visual pigments have broad spectral sensitivities, only three or four pigments are useful for the extraction of spectral information from the environment, but that if the sensitivities of the photoreceptors are made narrower by optical filters then more detailed spectral information could be obtained using an increased number of channels. Cronin and Marshall suggest that mantis shrimps could take advantage of this and be able to discriminate among spectra of broadly similar shape: a capability that could be significant in intra-specific communication and the recognition of individuals.

This may be the case, but we are also able to detect some intraspecific colour differences in mantis shrimps with our own 'limited' trichromatic colour system. It is difficult to appreciate that these crustaceans could have an eight-dimensional colour space encompassing a similar spectral range to our own three-dimensional colour space. Most animals with tetrachromatic or potentially pentachromatic systems extend their sensitivity outside our spectral range into the ultra-

tion of light⁴ and contain only a single visual pigment with peak sensitivity at 510 nm. The regions of the eye both dorsal and ventral to the equatorial band also contain only a single visual pigment with maximum sensitivity at 498 nm (ref. 3).

It is rows 1 to 4 of the central band that contain the striking 'colour' vision system



Compound eye of *Odontodactylus scyllarus*.

of the shrimp. Here the rhabdomeres form two tiers, and in rows 2 and 3 the tiers contain brightly coloured intra-rhabdomeral filters. In row 2 the filters are

violet and/or the infrared^{2,8}. The exact function of the complex of photoreceptors in the mantis shrimp can be determined only if we know how the eight classes of receptor are neurally integrated. Do the four ommatidial rows act as simple dichromatic systems, each covering only part of the shrimp's visible spectrum? Do rows 1 and 2 and 3 and 4 work as two parallel pairs because both pairs, at least to some extent, duplicate each other's spectral sensitivity? Only detailed behavioural and electrophysiological investigations will suggest answers to some of these questions. As with any novel system, its description raises more

questions than answers, and we are still left with the question: what kaleidoscope of colours does a mantis shrimp see? □

J.K. Bowmaker is in the School of Biological Sciences, Queen Mary College, University of London, Mile End Road, London E1 4NS, UK.

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MOLECULAR PHYLOGENY

Archaeobacteria and Archezoa

T. Cavalier-Smith

LONG after Darwin first urged us to base biological classification on genealogy, the largest classificatory units are still controversial. But these recent controversies result from rapid progress, not stagnation. It was two centuries after Linnaeus put bacteria in the animal kingdom and fungi in the vegetable kingdom that electron microscopy and molecular biology established the more fundamental divisions between viruses and cellular organisms, and within the latter between prokaryotes (bacteria) and eukaryotes (those with cell nuclei). For the past decade, the question has been how prokaryotes and eukaryotes should be subdivided into kingdoms. This is illuminated by three papers comparing

ribosomal (r) RNA sequences: Gouy and Li on page 145 of this issue¹ deal with the primary subdivision of bacteria, and Sogin *et al.*² and Perasso *et al.* on page 142 of this issue³ clarify the primary divisions within eukaryotes.

In prokaryotes, the main subdivision is no longer between 'blue-green algae' and 'bacteria' but between Archaeobacteria, which have cell membranes made of unusual isoprenoidal ether lipids, and Eubacteria, with membranes of typical fatty acid ester lipids like those of eukaryotes. The photosynthetic 'blue-green algae', now renamed Cyanobacteria, are merely one of about ten eubacterial phyla. Archaeobacteria fall into three distinct groups: Methanobacteria, which get energy by converting carbon dioxide and hydrogen to methane; Halobacteria, which thrive in hypersaline waters; and Sulfo bacteria which depend on sulphur compounds for energy.

Although many biologists accept the validity of the kingdom Archaeobacteria, the classification has been questioned by Lake⁴, who argues that Sulfo bacteria (which he calls 'eocytes') are more closely related to eukaryotes than to other Archaeobacteria; that Halobacteria, on the other hand, are closer to eubacteria than to other Archaeobacteria; and, therefore, that Archaeobacteria should be split into two kingdoms. Initially, Lake's proposal was based on somewhat tenuous evidence, but it seemed to gather strength when he devised an ingenious new method for calculating phylogenetic trees from sequences, which he calls evolutionary parsimony, and applied it to the small subunit (ss) rRNA.

Guoy and Li¹ in their new work confirm Lake's conclusion that this method gives a tree that links Sulfo bacteria more closely to eukaryotes than to other Archaeobacteria. By contrast, earlier trees obtained by Woese using a distance-

matrix method, on which the idea of Archaeobacteria was founded, show Sulfo bacteria, Methanobacteria and Halobacteria as sub-branches of a single archaeobacterial branch of the universal tree. Gouy and Li¹ try to show which method gives the right answer. The distance-matrix method is relatively straightforward: a tree is calculated with branch-lengths proportional to the degree of difference between the aligned DNA sequences of the species included (Fig. 1). Because mutations from one base to another are reversible, repeated changes at one site in the DNA can return it to its original state and lead to underestimates of the actual change that get worse with the degree of divergence. Although this bias is corrected statistically before calculating the tree, the calculated branch-lengths depend on an average of several such 'corrected' differences and are only approximate. If these errors are comparable to the branch-lengths themselves, then the branching pattern can be wrong.

The seriousness of these errors depends partly on the branching pattern itself; a tree with well-spaced branches is inherently more robust than a bush with numerous branches emerging almost together — for such a bush one must be sceptical of assertions about the order of branching of different lineages. The problem is worse the shorter the sequences being studied; thus trees based on short molecules such as transfer RNA (about 70 nucleotides) or 5S rRNA (about 150 nucleotides) are notorious for their unreliability — usually producing a mixture of biological sense and nonsense. Using ss rRNA (about 1,400–2,200 nucleotides) greatly reduces (but does not eliminate) this error; for eukaryotes the ss rRNA distance-matrix trees agree remarkably well with those based on cell ultrastructure using cladistic arguments (where organisms sharing complex evolutionarily derived characters are grouped together), which gives considerable confidence in both methods.

Because the large ribosomal subunit (ls) rRNA is twice as long as ss rRNA, trees based on this molecule should be even less prone to random error. Gouy and Li¹ therefore use ls rRNA data to make trees, and also combine the ss and ls rRNA to yield composite trees. They find that a distance-matrix method (neighbour joining) gives exactly the same result as it does for the ss rRNA, that is, all Archaeobacteria are connected together in a single branch, as Woese found. Moreover, in both cases they also obtain this same tree using evolutionary parsimony. Because a third, independent method, maximum parsimony, which chooses the tree topology giving the smallest total number of mutations, also shows Archaeobacteria as a single branch, it is highly probable that Lake's ss rRNA evolutionary parsimony

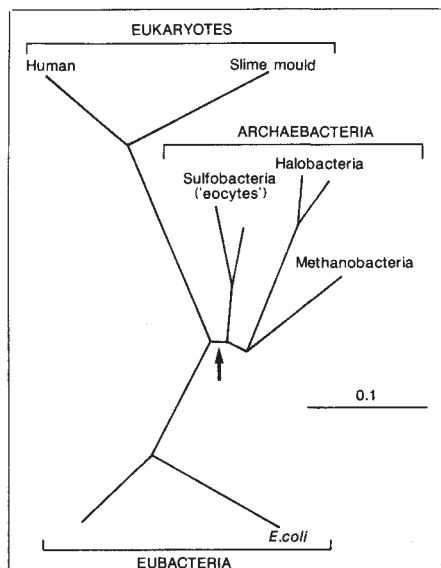


FIG. 1 Universal evolutionary tree based on combined ss and ls rRNA sequences. The tree has no root because from rRNA sequence data alone one cannot determine where it should be. Redrawn from ref. 1 with branches proportional to the degree of sequence divergence (scale indicates the fraction of nucleotides changed).

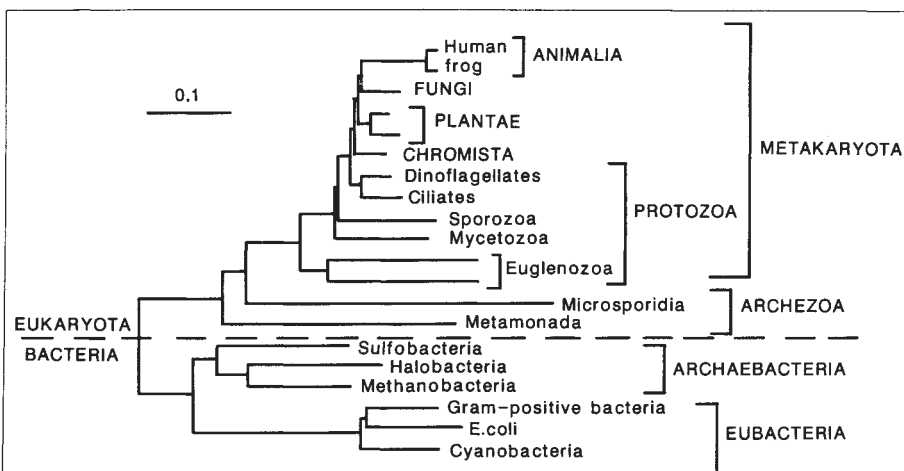


FIG. 2 Universal evolutionary tree based on ss rRNA sequences. The scale shown represents 10 changes per 100 nucleotides; the horizontal lengths only of the tree are meaningful. (Redrawn from ref. 2 and relabelled; courtesy of M. L. Sogin.)

tree is incorrect. This error may have arisen because evolutionary parsimony (which works by calculating mathematical invariants for alternative trees) uses less than half as many mutational differences as the other methods, which is equivalent to using shorter molecules with relatively greater random errors. This would be most likely to cause problems in the archaeobacterial branch of the tree, where, as Gouy and Li show, the initial segment at its base (arrow in Fig. 1) is scarcely longer than the standard error.

A similar problem probably caused another apparent treeing contradiction⁵ concerning *Prochlorothrix*: in that case the 16S rRNA distance-matrix trees are statistically robust but the *psbA* parsimony tree is not, because a trivial variant of the analysis gave a result indistinguishable from the 16S rRNA tree.

For bacteria, the primary division into kingdoms Archaeobacteria and Eubacteria was originally suggested by their divergent rRNA sequences and then confirmed by other characters such as different membrane lipids. For eukaryotes, however, the reverse has been true; ultrastructural and organismic properties were the original basis for proposing a primary subdivision into two groups: a kingdom Archezoa⁶ comprising four phyla that totally lack mitochondria and peroxisomes; and a superkingdom Metakaryota⁷; only later are ss rRNA sequences becoming available in support of this division.

Following earlier evidence from Woese's group⁸ for a very deep divergence between one archezoan phylum (Microsporidia) and metakaryotes, Sogin's group² now shows that a second archezoan phylum (Metamonada, represented by a new sequence from *Giardia*) diverged even earlier (Fig. 2). Moreover the *Giardia* ss rRNA sequence is primitive in that it contains many more sites identical to those of bacteria (particularly Archaeobacteria) than does any other eukaryotic sequence. The deep divergence between

Archezoa and Metakaryota is greater than that between Archaeobacteria and Eubacteria, and clearly supports the separation of Archezoa at least as a separate kingdom; though Sogin *et al.*², curiously, suggest that eukaryotes should all be lumped into a single kingdom. Evidence is accumulating that the size of archezoan ss rRNA, ls rRNA and ribosomes are 16S, 23S and 70S, respectively, as in bacteria, rather than 18S, 28S and 80S, as in metakaryotes. Several features that have been assumed to be true for all eukaryotes may turn out to apply only to metakaryotes and not to Archezoa.

Although the fundamental gulf between Archezoa and metakaryotes is increasingly obvious, there are still too few complete rRNA sequences to resolve the controversies over the subdivision of metakaryotes: in the absence of full sequences, even partial ones can be valuable. Thus Perasso *et al.*³ in their paper in this issue report the use of 450 nucleotides at one end of the ls rRNA to obtain approximate positions for various algae. Their most significant result con-

cerns red algae, which before acceptance of the symbiotic origin of chloroplasts were often thought to be the most primitive eukaryotes. These organisms are now clearly shown to be relatively advanced, grouping with green plants and animals rather than with primitive protozoa or Archezoa. Their results are also significant for the Chromista⁹ (a kingdom of mostly photosynthetic, mostly brown eukaryotes with chlorophyll-c-containing chloroplasts inside their rough endoplasmic reticulum); they confirm a relatively deep split, as predicted on ultra-structural grounds⁹, between the sub-kingdoms Cryptomonada (having phycobilin pigments) and Chromophyta (lacking them).

Because of the short sequences determined in this work³, relatively minor features of the tree of Perasso *et al.*, such as the grouping of red algae with cryptomonads, the separation of cryptomonads and chromophytes, the grouping of haptomonads with the other chromophytes, and the grouping of fungi and chromophytes, should not be taken too seriously, especially as the last is contradicted by Sogin's full ss rRNA trees. It will require at least complete ss rRNA sequences for these groups, and probably in some cases also combined ss and ls rRNA sequences as in Gouy and Li's analysis, to show which, if any, features are correct. □

T. Cavalier-Smith is a professor and fellow of the Canadian Institute of Advanced Research in the Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 2B1.

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STEREOPSIS

Vision of solid objects

Michael J. Morgan

WHY do we have two eyes, both facing forwards? One answer might have been given by the Cyclops: loss of a single eye would be too catastrophic. But if we are to have two eyes, there would be much to be said for placing them on opposite sides of the head as the rabbit does, where they would provide panoramic vision. Our own left and right eyes examine the same region of the world. Ever since Wheatstone's discovery of the stereoscope, it has been widely assumed that the advantage of two forward-facing eyes is to provide stereoscopic vision. Because the two eyes view the same objects from a slightly

different perspective, the brain can use their different images to extract three-dimensional shape. Wheatstone showed that this binocular disparity is sufficient to yield an impression of stereopsis: literally, 'solid vision'. On page 135 of this issue¹, Rogers and Cagenello provide support for the idea that binocular disparity gives accurate information about shape, and they suggest a way round one of the main problems that the visual system faces in constructing shape from disparity information.

The problem in constructing shape from disparity is that disparities change with

Sir Harold Jeffreys (1891–1989)

SIR HAROLD JEFFREYS, who died on 18 March, was one of the main architects of modern geophysics. In the distinguished English tradition of applied mathematics, he established with contemporary seismologists (B. Gutenberg and I. Lehmann) the structure of the Earth's interior, and with his student (K.E. Bullen) provided in their seismic-travel-time tables an indispensable tool for research. Although the achievement was based on the simple laws of classical physics (newtonian mechanics, elasticity and heat conduction), he was a pioneer in searching for evidence in the Earth's mantle, the Moon and the satellites, of departures from perfect elasticity. This enabled him to explain why Earth models derived from the free oscillations of the Earth, discovered when long-period seismometers were installed, differed slightly from those based on observations of body waves.

Jeffreys contributed greatly to the discussion of astronomical data: to the precession of the Earth and to the slowing down of its rotation by tidal friction. A pioneer in what is now called comparative planetology, Jeffreys, by analogy with the Moon's figure, showed by spherical harmonic analysis of values of the acceleration g due to gravity over land and ocean that the Earth also departs from the hydrostatic model over long wavelengths. Although this result was not believed, Jeffreys was proved correct when the geoid was determined by Earth satellites. The geoid is now accepted as evidence for solid-state convection in the Earth's mantle — a remarkable paradox as Sir Harold's opposition to continental-drift theory was legendary.

He was sceptical of the qualitative

evidence for Wegener's drift theory, for his aim, cogently expounded in seven editions of his great treatise *The Earth* was to provide a secure mathematical basis for his subject. When quantitative evidence came for Wegener's theory, it was in a field — geomagnetism — which Jeffreys had not supposed to be relevant to an understanding of the Earth's mechanics. Moreover he had been the leading advocate of the Earth-contraction theory of mountain building, which had been almost universally believed by geoscientists until the great conversions of the mid-1960s to drift models. But Sir Harold, who thought deeply about scientific inference, once remarked "there is no such thing as a final scientific theory".

Sir Harold and Lady Bertha Jeffreys' *Methods of Mathematical Physics* was greatly valued by students, although as a lecturer today, Jeffreys would scarcely have survived the British government's teaching-assessment schemes naively designed to 'improve higher education'. Yet he inspired generations of young geophysicists — and not only by his writing.

Sir Harold was born at Birtley, and educated at Rutherford (Grammar) School and Armstrong College (now the University of Newcastle upon Tyne) and remained throughout his life devoted to Northumberland and County Durham. A Fellow of St John's College, Cambridge, for almost 75 years and a Fellow of the Royal Society, from which he received a Royal and the Copley Medals, for 64, he remained actively interested in geophysics and kind and encouraging to young geophysicists.

Keith Runcorn

Keith Runcorn is Professor of Physics at the University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK.

viewing distance. It is intuitively obvious that the further an object is away, the more similar its images become in the two eyes. (For the same reason, stellar parallaxes are difficult to measure accurately.) In fact, the difference in angular separation between two points in the left and right eyes (their disparity) decreases as the square of the viewing distance. We cannot, then, use disparity by itself as a cue to the difference in distance of two objects. The same argument applies to two points on the surface of a single object, and thus to the three-dimensional shape of that object. Despite this, the perceived shape of an object such as a football does not greatly change with viewing distance. How does the visual system achieve this remarkable feat of shape constancy?

What shape constancy requires is a property of disparity which, unlike absolute differences in disparity between points (the disparity gradient), is invariant with viewing distance. Disparity curvature, the second differential of disparity

over space, fulfils this requirement². The second differential of disparity represents the rate at which the gradient of disparity is changing, or in other words the acceleration of disparity. Although the disparity gradient represents the difference in disparity of a point from a neighbouring point, the disparity curvature represents the difference in gradient between adjacent pairs of points. In the case of a flat surface tilted in depth, for example, the disparity curvature will be zero at whatever viewing distance, despite changes in the disparity gradient with distance. The disparity curvature is a property of the surface, which does not change with viewing distance.

If the visual system does indeed extract disparity curvature, it would be using a strategy that has apparently been found useful in other contexts. Ernst Mach proposed that at any early stage in visual processing, the perceived brightness of points in the retinal image is represented by the second derivative of their physical

intensity values, thus accounting for the appearance of 'Mach bands' at points on intensity luminance profiles where the gradient changes. It is now widely accepted that an approximation to the second derivative of luminance could be extracted by neural receptive fields which subtract light in their centre from light in their surround. But it is far less obvious what the equivalent operation for calculating disparity curvature might be.

Following a similar suggestion by Koenderink³, Rogers and Cagenello in their paper in this issue suggest a simple algorithm for computing disparity curvature. To have a visible shape, an object must have markings on its surface. Even if these markings are straight lines on the surface (geodesics) they will have a two-dimensional curvature in the retinal image. The difference in curvature between the two eyes is related to the three-dimensional curvature of the surface and thus to the disparity curvature. The suggestion is that curvature disparity could be used as an approximation in computing disparity curvature, and thus three-dimensional shape.

Rogers and Cagenello report experiments in which observers judged the shape of objects defined by disparity information alone. In agreement with other work⁴, they find that observers can discriminate very small differences in curvature. Their most important result is that observers can accurately match the perceived curvature of two parabolic surfaces at different distances. Moreover, experiments manipulating the surface markings on objects showed that observers were most accurate at shape judgments when the orientation of the surface markers was such as to maximize their curvature disparity.

The curvature-disparity conjecture is not incompatible with other suggestions about the way in which raw disparities could be used to derive shape information^{5,6}. The visual system is never one to miss a good trick and it may well combine different sources of information such as curvature disparity and vertical disparities. The real world is much richer in information than the laboratory stereoscope, which may explain why there are some conditions in which shape can appear distorted when defined by disparity alone⁴. If curvature disparity is indeed used in vision, this may explain why observers are so good at extracting two-

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dimensional curvature⁷ and why curvature-tuned receptive fields apparently exist in the visual system⁸. In the absence of direct comparisons of their efficiency, however, it would be premature to conclude that the mechanisms of two-dimensional and three-dimensional curvature discrimination are the same. Earlier suggestions that precise two-dimensional shape judgements evolved for the purposes of stereopsis do not withstand critical analysis⁹.

A final thought on stereopsis is that it is likely to have several functions, including precise eye-hand coordination. The binocular reader who doubts the biological importance of stereoscopic information is invited to try collecting blackberries with

one eye shut. The decreased performance level will be measured both by the decreased yield of fruit and the increased number of scratches. In normal reaching behaviour we have binocular vision not only of the object we are reaching for, but also of the hand. In these circumstances, it is not clear that the primary goal of stereopsis should be the extraction of absolute rather than relative distance information. Extraction of shape may be an additional bonus of stereopsis, rather than its primary function. □

Michael J. Morgan is Professor of Psychology at University College London, Gower Street, London WC1E 6BT, UK.

SOLAR SYSTEM

Meteorites large and small

D.E. Brownlee

INTERPLANETARY dust particles and meteorites yield invaluable insights into the processes, materials and environments that existed during the earliest history of the Solar System. Conventional meteorites are wonderful samples but they provide an incomplete perspective of the comets and asteroids that have survived as relic planetary building materials. Dust grains, by contrast, provide a wider range of origins. On page 126 of this issue¹, Klöck *et al.* report analyses of unusual manganese-rich olivine and pyroxene grains that provide an interesting means of exploring possible links between materials in conventional meteorites and interplanetary dust particles that may be of cometary origin.

As recently discussed by R.T. Dodd in *News and Views*², the thousands of conventional meteorites are probably fragments of only a small number of asteroids with no cometary representatives. Spectral-reflectance studies indicate that meteorites are not representative of typical asteroids nor do they reflect the range of materials seen in the asteroid belt. For example, the primitive 'D-class' objects that are abundant among the outer asteroids are composed of dark anhydrous materials³ that have no meteoritic analogues. Selection effects associated with gravitational perturbation to Earth-crossing orbits and the strength required to survive atmospheric entry have produced a strong bias that has apparently prevented all comets and most asteroids from producing conventional meteorites.

In contrast, interplanetary dust particles (IDPs) potentially are derived from a broad range of both asteroids and short-period comets. Typical IDPs collected from the stratosphere are 5–50 µm in size and such small particles are not significantly affected by the orbital and strength constraints imposed on conventional

meteorites. Dust particles do not have to be strong to survive aerodynamic deceleration and, in space, the drag component of light pressure causes the orbits of all asteroid and cometary dust to spiral towards Earth-crossing orbits.

Using a transmission electron microscope, Klöck *et al.* have performed minor-element analyses on small olivine and pyroxene crystals contained in 0.1 µm thick microtome sections cut from individual chondritic-composition IDPs only 10 µm in size. Over half of the particles studied contained forsterite and enstatite grains with unusually high MnO contents above 0.5 per cent. The highest MnO content was 5 per cent with a Mn/Fe ratio enhanced above the solar ratio by a factor of over 200. The authors name these unusual grains LIME (low-iron, manganese-enriched) because they are distinctly more Mn-rich than their (sub-LIME) counterparts commonly found in chondrites⁴. In the meteorites examined, abundant LIME grains were found only in the fine-grained matrix of the Semarkona unequilibrated ordinary chondrite.

Similarities between this chondrite and a class of IDPs are of particular interest because Semarkona is one of the least metamorphosed ordinary chondrites and it contains smectites, carbides⁵ and enhanced deuterium in abundance as do some classes of interplanetary dust. The LIME grains as well as other minor- and trace-element anomalies in meteorite and IDP forsterite grains⁴ provide interesting ways of comparing primitive Solar System materials, but it is not currently possible uniquely to interpret the meaning of similarities. If components such as the LIME grains are produced only in a specific region of the solar nebula then their presence in both cometary and asteroidal materials would be evidence for mixing over a large radial distance scale. If

radial mixing is not important and the LIME grains are specific to a process, such as condensation, then the process apparently occurred over a wide radial range. Klöck *et al.*¹ suggest that condensation is a probable origin for the LIME grains because it provides a means of decoupling the incorporation of Fe and Mn into the crystals and enhancing the Mn/Fe ratio. Manganese can be stably incorporated into forsterite at a temperature only a few hundred degrees below the forsterite condensation temperature. Iron, on the other hand, should condense as metal at these temperatures and be essentially excluded from silicates. The high Mn and variable Mn/Fe ratios in LIME grains contrast the behaviour of Fe and Mn in igneous meteorites. In these meteorites, Mn and Fe are highly correlated because Mn freely substitutes for Fe owing to the similarity of the ionic radius of Fe²⁺ and Mn²⁺.

If some interplanetary dust particles could be positively identified as cometary then their properties, such as content of LIME grains, and the properties of asteroidal IDPs and conventional meteorites would provide constraints on regions of the solar nebula where asteroids and short-period comets formed. The asteroids formed in the transition zone between the terrestrial and giant planets; the short-period comets presumably formed an order-of-magnitude farther from the centre of the Solar System, either in the Uranus–Neptune region or in the 'Kuiper belt' just beyond the outer planets. The nature of nebular solids could vary considerably over this range because of the pressure and temperature gradient in the solar nebula and also because inductive heating of small planetesimals may have had a strong radial dependence⁶. Additional variations could be due to differences in the survival efficiency of presolar interstellar grains. Although comet samples must exist in present collections of interplanetary dust, it has not yet been possible to positively identify them. Flynn⁷ and Sandford and Bradley⁸ now suggest that velocity-dependent effects associated with atmospheric entry could be used as possible criteria to distinguish comet and asteroid samples collected in the stratosphere. □

D.E. Brownlee is in the Department of Astronomy, University of Washington, Seattle, Washington 98195, USA.

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Black-footed ferret update

Robert M. May

MANY readers will have heard about the black-footed ferret, which used to be found over a fair fraction of North America (eating prairie dogs), then declined to the point where it was suspected of being extinct, and was found again near Meeteetse in Wyoming in 1981. Soon after its discovery, the Meeteetse colony was devastated by an outbreak of canine distemper. By October 1985, this only known colony of ferrets had been reduced to six individuals who — after some controversy — had been taken into captivity by the Wyoming Game and Fish Department, along with six or so individuals remaining in the wild^{1,2}. *The Black-footed Ferret Recovery Plan*, just published by the US Fish and Wildlife Service³,

gating this species in captivity. By late 1988, the Wildlife Research and Conservation Education Centre at Sybille, Wyoming, was home to no fewer than 58 ferrets, 17 wild-caught and 41 captive-reared. The most immediate aim of the recovery plan¹ is to build the captive population to around 200, a goal which should be reached by 1991. By the year 2010, it is hoped to have established 10 or more separate populations in the wild, each numbering around 150 ferrets. This number — a total of 1,500 distributed among 10 or so subpopulations — was arrived at in a necessarily tentative way as offering a good chance of self-sustaining persistence in the face of such natural adversities as epidemics of disease and other environmental unpredictabilities^{5,6}.

A population of this size would also retain something like 90 per cent of the present genetic diversity in black-footed ferrets⁶, which itself is, however, significantly diminished by the bottleneck through which the population has just passed. A classic calculation of population genetics suggests that exchange of genetic material at a rate roughly corresponding to transferring one individual from each subpopulation to another, in each generation, will be sufficient to prevent the local subpopulations diverging genetically^{1,6}. It seems advisable to maintain such artificial manipulation of gene flow, at least until ferret populations are so widely distributed as effectively to constitute a large, contiguous population.

Not to be unrelievedly cheerful, this rescue mission cost about two million dollars up to 1986, with the money coming both from government agencies and from the private sector¹. Although this is a trifling sum compared with that spent on other scientific endeavours (how about sequencing the black-footed ferret genome; that should see them safe?), it is large by usual standards for this sort of endeavour. Conservation biology urgently needs funding at higher levels than it has heretofore received, or else Noah's Ark II is going to be rather empty. □

Robert M. May is a Royal Society Research Professor at Imperial College London and at the Department of Zoology, University of Oxford, Oxford OX1 3PS, UK.

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Ardea

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REASONS

Optimism for the black-footed ferret? offers a carefully considered view of how best to manage this threatened ferret population, in both the short and long run.

When I last wrote in *News and Views* on this topic³ early in 1986, a breeding programme was about to start within this captive colony of four females and two males. I took a pessimistic view. Subsequent developments at first were indeed gloomy: only one of the males appeared capable of breeding, and no offspring were produced in the captive-breeding programme during that winter and spring.

In the summer of 1986, however, five adults and two litters were found in the wild. One male ferret was captured and brought into the breeding population. Opinion continued to harden that the population was at risk of extinction, and that the only sensible action was to capture all the remaining wild animals. In September, most of the remaining wild ferrets were trapped, bringing the captive total to 17 (11 females and 6 males); one additional wild male was captured in February 1987^{2,4}.

The subsequent story is a happy one¹. Two litters were born in summer 1987, representing the first success at propa-

Maidenhead revisited

LIKE all vertebrates, human beings have two independent gonads: ovaries in women, testicles in men. This doubling-up of important organs is typical of nature's shrewd redundant design; somebody who loses one of the pair can still function quite well on the other. Our gonads remain dormant throughout childhood, and spring into action together at adolescence. Later on in life, as their owner's chances of further successful parenthood begin to fade, they decline together as well.

Daedalus now plans to dodge this melancholy but universal fate. Suppose, he says, that one gonad of the pair could be protected from adolescent maturation by some sort of local implant to its blood supply, absorbing or neutralizing the hormonal stimuli. It would then remain dormant while its partner came to life. The individual thus treated would grow up quite normally, and could enjoy an active and vigorous sexual life. But when at last middle age or menopause began to threaten, he or she would have the implant removed. The long-dormant second gonad would then blaze into action, resetting the biological clock right back to first youth.

Sadly, the precise hormonal triggering of puberty is still obscure. But DREADCO's biochemists, building on their recent search for a puberty-delaying hormonal antagonist, are confident of ultimately synthesizing the implant compound they already refer to as 'The Second Barrel'. Once perfected, it will revolutionize human life.

Firing the Second Barrel will have rapid and dramatic results. Adolescent vigour and sexual energy will blaze again in middle age. At first Daedalus had bizarre visions of fist-fights breaking out in Senior Common Rooms, of street gangs with names like Hell's Managers and Boot Boffins, of portly gentlemen in leather jackets chatting up giggling matrons on street corners while baffled teenagers looked on furiously at this invasion of their social niche. But this is too pessimistic. Second Barrellers will combine the vigour of youth with the wisdom and experience of age; they will handle their new powers with enviable grace and effectiveness. Their intellectual drive will also revive. A lifetime's knowledge, fanned anew by adolescent curiosity, vigour and alertness, should burst into fresh creative flame.

Women in particular will benefit from Second Barrel technology. Their implant will be more invasive but less noticeable than that for men. It will probably not extend fertility to an inconvenient age: the newly activated ovary will contain ova too old and decrepit to be viable. Far more effective and long-lasting than hormone replacement therapy, it will deliver in reality that enduring youthfulness that cosmetics only promise.

David Jones

Explanations of cold fusion

SIR—I suggest that electrolysis of a heavy-water solution of deuterated lithium hydroxide with a palladium cathode leads to the formation of a higher deuteride of palladium, probably PdD_3 with the fluorite structure, the subsequent decomposition of which leads to the increased evolution of heat or to the explosion of the deuteride into palladium powder and hydrogen gas.

In a paper in *Physical Review* in 1938 I pointed out that in the transition metals 0.72 orbital per atom of the orbitals in the outer shell remains apparently unused. Later, I identified the 0.72 orbital per atom as the metallic orbital, required for the unsynchronized resonance of covalent bonds that confers metallic properties on a substance. The principal evidence for the metallic orbital is that the curve of the saturation paramagnetic moments of the iron-group metals has a foot at the composition $\text{Ni}_{0.4}\text{Cu}_{0.6}$. I also pointed out that the need for the metallic orbital for stability explains the fact that palladium is saturated with hydrogen at the composition $\text{PdH}_{0.6}$. Introduction of additional hydrogen atoms leads to instability because of the loss of resonance energy when the metallic orbital is not available.

I judge that under the conditions of the electrolysis experiments of M. Fleischmann and S. Pons (*J. electroanal. Chem.* **261**, 301–308; 1989) deuterons beyond this limit are forced into the palladium cathode, ultimately forming the unstable higher deuteride. Later, after the beginning of electrolysis, this unstable deuteride may begin to decompose either slowly, resulting in an increased liberation of heat, or explosively, as Fleischmann and Pons observed when a 1-cm cube of the deuterated palladium disappeared.

It can be predicted that, because of the difference in amplitude of the zero-point vibrations of the nuclei with different masses, palladium dihydride would be less stable than palladium dideuteride, and palladium ditritide would be more stable — perhaps stable enough to permit examination by X-ray diffraction.

LINUS PAULING

Linus Pauling Institute of Science
and Medicine,
440 Page Mill Road,
Palo Alto, California 94306, USA

SIR—Hydrogen in metals may tend to segregate to cracks, grain boundaries or interfaces and to dislocations. This is a well-known phenomenon and it explains several important observations such as hydrogen-induced fracture in ferritic steels. We suggest that prior accumulation of hydrogen in defects in palladium electrodes may prevent the build-up of deuterium, and that this could explain why cold fusion is hard to replicate.

The equilibrium segregation of hydrogen can be characterized as a function of the binding energy and of the local stress for the special case of a crack. The quantity of segregated hydrogen, $(C_H)_i$ is given by

$$(C_H)_i = \frac{C_m \exp((\Delta H_B)_{ef}/kT)}{1 - C_m + C_m \exp((\Delta H_B)_{ef}/kT)}$$

For a surface, grain boundary, interface or dislocation, the effective binding energy of hydrogen is $(\Delta H_B)_{ef} = (\Delta H_B)_j$, whereas for a crack-tip it is $(\Delta H_B)_{ef} = (\Delta H_B)_j + \sigma_j V_H$. In these equations, the binding energy is ΔH_B and the local stress is σ_j . The subscript j indicates the segregation site of the hydrogen (for example, s for crack surfaces and b for boundaries). C_m is the bulk hydrogen content, V_H is the molar volume of hydrogen, k is Boltzmann's constant and T the absolute temperature. $(\Delta H_B)_j$ can be described by the standard chemical potentials of hydrogen and the metallic matrix in which it segregates, at the segregation site and remote from it. Thus the magnitude of $(\Delta H_B)_j$ depends on the type of hydrogen segregation site¹.

We suggest that when in a cold fusion experiment a palladium electrode is immersed in a heavy water-based electrolyte, the deuterium diffuses into the palladium and segregates to dislocations and other defects according to the above equation. When the concentration in these local regions has reached a high enough level, the reaction commences in the segregated region. We are unable to say just what the reaction is, but it may be a form of nuclear fusion or some chemical reaction. The period of electrolysis that precedes the reaction is attributed to the build-up of deuterium to the critical concentration in the regions around the dislocations or other defects in the palladium.

If, during the period before the experiment commences, hydrogen has diffused into the palladium and segregated to the dislocations, there will be fewer lattice interstitial sites into which the deuterium can migrate when electrolysis in heavy water commences, and the deuterium concentration around the defects may never attain a high enough level for the 'fusion' reaction to start.

If this hypothesis is correct, it accounts for the failure of some laboratories to produce the 'fusion' reaction described by Fleischmann and Pons². The solution is to purify the palladium before electrolysis so as to remove hydrogen and any other interstitial atoms that, by segregating to the crystal defects, prevent the critical

concentration of deuterium from being attained in the experiments.

JOHN GITTUS

UK Atomic Energy Authority,
11 Charles II St, London SW1Y 4QP, UK
JAMES BOCKRIS

Texas A&M University,
College Station, Texas 77843, USA

Stereo-image

SIR—Vance Tucker (*Nature* **337**, 605; 1989) notes that few readers keep stereoscopes handy and that only a quarter of those he surveyed could view stereo-pairs without one.

The main function of a stereoscope is to counteract the normal correlation between the focus and convergence muscles in the eye. Its lenses bring the focus to the printed page while the eye muscles are focused and converged at infinity, with the left eye viewing the left-hand image, and the right eye the right.

On the other hand, I wear glasses for near-sightedness to bring objects at infinity into focus, as my unaided eyes' maximal focus distance is much closer. Thus I and other near-sighted readers can create a stereoscope by merely removing our glasses.

NELSON MAX

3-28-8-412 Yaguchi,
Ota-ku, Tokyo 146, Japan

Trilobite attacks

SIR—Babcock and Robison¹ report that sublethal predation scars on Palaeozoic trilobites occur about three times as frequently on the right side of the trilobite body as on the left. They conclude that the predators of the period apparently preferred to attack the trilobite's right side, and that this provides evidence of behavioural asymmetry and brain laterality in the fossil record. I would like to suggest an alternative explanation, related to the trilobite's defensive response to attack.

Many trilobite fossils are found curled up². Curling probably enabled trilobites to protect their more vulnerable ventral surface, in a manner perhaps analogous to that of modern-day terrestrial isopods such as garden slaters (woodlice or sow bugs). Picture the following. Before the trilobite completes the curl, the middle lateral margins are raised off the substrate as the head (prosoma) or tail (pygidium)³ is curled beneath the body. Since the head shield provides the greatest protective surface, curling would probably have started at the tail. A balanced, bilaterally symmetrical, curling motion would simultaneously lift both lateral margins off the substrate as the body is arched so that sublethal predation scars would be expected to be equally frequent on both sides. This would also be the case if the trilobite toppled over onto one side as curling

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2. Fleischmann, M. J. & Pons, S. J. *electroanal. Chem.* **261**, 301–308 (1989).

progressed (toppling to the right or left would be random due to the external bilateral body symmetry).

The results of Babcock and Robison, however, suggest that curling was not bilaterally symmetrical but skewed to favour retraction on the animal's left, thus raising and exposing to attack the right-hand margin of the trilobite's body.

If curling did indeed commence at the tail-end and was skewed to favour retraction on the animal's left, then the right-hand margin of the pygidium and posterior thoracic segments would have been exposed first and for the longest period. One might therefore expect to see a higher frequency of sublethal predation scars in these areas. Interestingly, the three examples shown by Babcock and Robison are all of injuries to the right-hand region of the pygidium and/or posterior thorax.

DAVID P. MAITLAND

Department of Physiology,
Medical School,
The University of the Witwatersrand,
Johannesburg, South Africa 2193.

SIR—Babcock and Robison show that for their sample of Palaeozoic trilobite fossils, there is a marked tendency for healed injuries to occur on the right side rather than the left, and they interpret this as evidence of behavioural asymmetry in the fossil record, support for an early evolutionary history for brain laterality¹. I would suggest an alternative, simpler explanation. Trilobites may have possessed a biological asymmetry that put a soft vital organ on their left side, as in the case of the human heart, making attacks to that side more likely to be fatal. Simply put, it may be that trilobites were attacked equally often on both sides, but more of those attacked on the right side survived to join Babcock and Robison's sample.

An example of such a phenomenon occurred during the Second World War. The US military studied fighter planes returning from missions to try to improve their survival rate and were considering adding heavy armour to those parts of the plane that tended to show the greatest concentration of hits from enemy fire, until statisticians pointed out the fallacy of that argument². The more vulnerable parts of the plane were those with the fewest hits; planes hit there tended not to return at all. The single most vulnerable part, the pilot's head, was without serious scar in the sample of planes that returned.

STEPHEN STIGLER

Department of Statistics,
University of Chicago,
5734 University Avenue,
Chicago, Illinois 60637, USA

1. Babcock, L.E. & Robison, R.A. *Nature* **337**, 695–696 (1989).
2. Meglitsch, P.A. *Invertebrate Zoology* (Oxford University Press, London, 1967).
3. Wallis, W.A. *J. Am. Statist. Ass.* **75**, 334–335 (1980).

Quasar lensing by galaxies?

SIR—We wish to point out that the extraordinary statistical alignments reported by Webster *et al.*¹ between low-redshift galaxies and high-redshift quasars, if explained as a gravitational lensing effect, can be used to place a robust lower limit on the total cosmological density. We also amplify a point raised by those authors, namely that, although the effect is expected to occur, the quantitative assumptions that one has to make to explain such a strong effect are inconsistent with our present understanding of galaxies. We thus disagree with Canizares' assessment²; in our opinion these alignments may indeed be "too close for comfort".

According to the lensing hypothesis, the images of high-redshift quasars experience an amplification when they are seen through foreground galaxies; this amplification allows one to see intrinsically fainter quasars, which are more numerous. Taking Webster *et al.*'s model at face value, suppose that there is an amplification of about a factor of two for the fraction $f \approx 0.01$ of the sky that lies within 6 arcsec of galaxies above their magnitude limit. The distance of galaxies at a redshift z is about $r = zc/H$, where H is Hubble's constant, and for their sample Webster *et al.* quote $z \approx 0.3$. Gravitational amplification by a factor of two with this geometry requires a mean surface density of lensing material of $\Sigma \approx 0.3g \text{ cm}^{-2}$, whether the lensing amplification is due to 'microlensing' by individual stars or to the galactic potential as a whole³. From this information alone we can estimate the mean density contributed by this lensing population; in terms of the cosmic density parameter, Ω ,

$$\Omega_l \approx \frac{8\pi G f \Sigma \theta^2}{H z c} \approx 0.15 \theta^2 h^{-1}$$

where θ^2 is the ratio of the actual lens area to the 6-arcsec circle, and $h = H/100 \text{ km s}^{-1} \text{ Mpc}^{-1}$; according to current estimates, h should lie between 0.5 and 1. Ω_l is a lower limit on the mean density contributed by material projected within about $16.5 \theta h^{-1} \text{ kpc}$ of a galaxy, for $z = 0.3$.

This density estimate is consistent with some other measurements of cosmic dark-matter density, but the dark matter here is much too close to the luminous parts of the galaxies to be consistent with other dynamical mass measurements. Based on estimates of the luminosity density of all galaxies, the mass-to-light ratio required to achieve the above mean density estimate is about $M/L \approx 240 \theta^2$ in solar units (independent of h). On no scale are dynamical estimates of mass⁴ consistent with such a large M/L . The range of M/L found for the inner parts of ordinary

galaxies ($\theta \approx 1$) is between 5 and 20, even allowing for the presence of a massive halo; on the scale of groups and clusters ($\theta \approx 20$) dynamical measurements range up to $M/L \approx 400h$, far short of what is required on this scale. On this and still larger scales, the density of the lenses is not consistent with limits on the overall deceleration of the Universe.

Unusual alignments or structures do not resolve this dilemma; if only a small fraction of galaxies contributes to the effect, then the quasar surface density enhancement required by the observations is correspondingly higher, and the problems get worse. Therefore, within a conventional understanding of galactic systems we can find no model to explain the large enhancement seen by Webster *et al.*

CRAIG J. HOGAN
RAMESH NARAYAN
SIMON D.M. WHITE

Steward Observatory,
University of Arizona,
Tucson, Arizona 85721, USA

WEBSTER *ET AL.* REPLY—We agree with the conclusions of Hogan, Narayan and White, that the alignments we reported¹ could suggest that the mass associated with galaxies is greater than has been previously estimated. However, the data in ref. 1 demonstrate a highly significant detection of an overdensity with a 2σ lower bound of 1.7. Estimating Ω_l using this lower bound significantly lowers the value calculated.

The calculated overdensity will vary with θ , and thus so too will estimates of the mass. Our data relate to the average overdensity for radii of 4–6 arcsec. We note that the surface mass density implied for the galaxies includes a contribution from any 'halo' component which may extend to many tens of kiloparsecs.

We are in the process of substantially increasing the sample size, obtaining galaxy redshifts, and obtaining CCD images of all quasar–galaxy associations. These data should greatly improve our estimates of the size of the effect and hence of the mass required for lensing.

RACHEL L. WEBSTER

Astronomy Department,
University of Toronto,
Toronto M5S 1A1, Canada

PAUL C. HEWETT
MARGARET E. HARDING

Institute of Astronomy,
Madingley Road,
Cambridge CB3 0HA, UK

GARY A. WEGNER

Dartmouth College,
New Hampshire 03755, USA

1. Webster, R.L., Hewett, P.C., Harding, M.E. & Wegner, G.A. *Nature* **336**, 358–359 (1988).
2. Canizares, C.R. *Nature* **336**, 309–310 (1988).
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4. Faber, S.M. & Gallagher, J.S. *A. Rev. Astr. Astrophys.* **17**, 135–188 (1979).

A natural selection

R. C. Lewontin

Evolutionary Genetics. By John Maynard Smith. Oxford University Press: 1989. Pp. 325. Pbk £16.95, \$32.50.

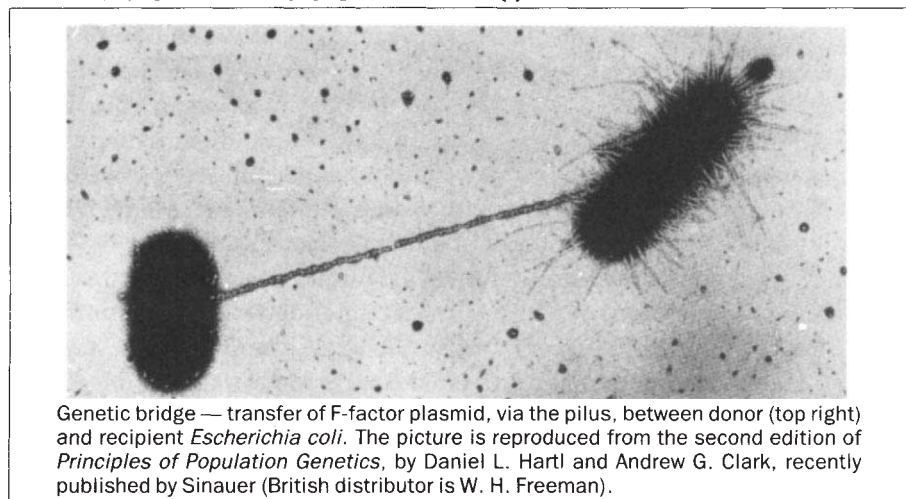
IN NOT paying more attention to textbooks, historians of science have missed an important source. When texts are written on general elementary subjects they reveal to us how changes in specialized knowledge have succeeded in permeating the unquestioned dogma of a subject. So, the frequent occurrence in general biology textbooks of ideas about sociobiology show that, whatever their shortcomings, sociobiological concepts have reached the status of received truth. When, on the other hand, a specialized subject is treated by an expert who is part of the research community of the field, the changes in the intellectual structure, the problems and the methods of the field can be seen. John Maynard Smith's new book is a model example.

The very title, *Evolutionary Genetics*, announces a transformed subject. Twenty years ago, when asked what I did for a living, I unhesitatingly said that I was a 'population geneticist'; now, when asked the same question, I say with some tentativeness, 'evolutionary geneticist'. The first textbooks of our field written 40 years ago by Lancelot Hogben and C.C. Li, and a later classic text such as Crow and Kimura's, all reflected a subject that was primarily concerned with the prediction of short-range changes in the frequency of genes in populations, or the equilibria of gene frequencies without any historical perspective. It was a kind of auto mechanics, concerned with carburettor settings and tyre pressures, but not with how the car was manufactured or where it was going.

Everybody agreed that population genetics was the basic theory on which evolutionary change was to be explained, but that relation was never 'cashed out', as philosophers are wont to say. But, over the past 20 years, the longer-term questions of evolutionary change have reasserted themselves as central, and the subject has been reorganized, partly under the impact of experimental and theoretical developments in molecular evolution, and partly as a consequence of the flowering of strategic analysis and optimality theory in biology.

John Maynard Smith has never been a 'population geneticist', *sensu stricto*, but with the catholicity of his mentor, Haldane, has for 40 years worked on developmental genetics, artificial selection, the theory of sex, evolutionary ecology, ageing and, as every school child knows, a special form of game theory applied to evolution and

behaviour. Especially with this last research he has played an important role in transforming 'population' into 'evolutionary' genetics, and the reorganization of the field is reflected in the organization of his book. In place of long initial chapters setting up the apparatus of random and selective mating, of the rococo intricacies of inbreeding and recombination, Maynard Smith introduces the concept of fitness by page 36 and by page 45 has



Genetic bridge — transfer of F-factor plasmid, via the pilus, between donor (top right) and recipient *Escherichia coli*. The picture is reproduced from the second edition of *Principles of Population Genetics*, by Daniel L. Hartl and Andrew G. Clark, recently published by Sinauer (British distributor is W. H. Freeman).

already dealt with the dynamics of spread of a new, favourable gene through a population. So, evolution has already occurred with only 15 per cent of the book gone. All the rest is commentary.

This organization reflects Maynard Smith's commitment to natural selection as the real stuff of evolution, despite occasional ceremonial bows to the forces of irrational chance. But he is not alone in this conviction. It is the great irony of modern evolutionary genetics that the spirit of explanation has moved more and more towards optimal adaptation, while the technical developments of population genetics of the past 30 years have been increasingly to show the efficacy of non-adaptive forces in evolution. There is indeed one chapter of 23 pages on the theory of neutral evolution. But the reader could hardly imagine the immense multiplication of explanatory approaches that have been developed in the past decades, including random fixation of deleterious genes, the random loss of favourable mutants, the stochastic drift of gene frequencies, genetic hitchhiking (Maynard Smith himself worked on this problem), developmental constraints and allometry, disadaptive frequency dependent selection, meiotic drive etc., etc. It is

not that these phenomena are not mentioned, but they are clearly diversions from the big event, the ascent of Mount Fitness by Sir Ron Fisher and his faithful Sherpas, Hamilton, Smith and Trivers.

The commitment to the adaptationist way of looking at things allows Maynard Smith to deal with a great variety of major evolutionary problems that the more mechanically minded population geneticist only finds an embarrassment. The second half of *Evolutionary Genetics* takes up such perplexing problems as the origin and maintenance of sex, of recombination, of genetic systems in general, the evolution of viruses and eukaryotes, and the evolution of cooperation, speciation and macroevolution. These are the questions that population genetics was supposed to deal with but could not

because of an obsession with detailed mechanism and a belief in the multiplicity of effective forces. By sweeping away the genetic details, as Maynard Smith's theory of evolutionary stable strategies and Lande's phenotypic treatment of morphological evolution have done, evolutionary genetics has almost ceased to be genetics at all. In this spirit, there are very few derivations of results in *Evolutionary Genetics*, and indeed remarkably little mathematics. In part, this is a reflection of the level at which the book is aimed; but, more deeply, it reflects the modern view that the details do not matter because, irrespective of the path, all roads lead to fitness. *Evolutionary Genetics* might better be named just *Evolution* and have done with it.

I am not sure that I want students to read *Evolutionary Genetics*, because it will do such a good job of teaching them a way of thinking about evolution with which I am not in sympathy. I am sure, however, that every population geneticist ought to read it, because it will tell them why so much of what they do seems unappealing to evolutionary biologists. □

R.C. Lewontin is a Professor in the Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138, USA.

C. C. Britton Jr & J. Carnahan

Star dust

David W. Hughes

Dust in the Universe. Edited by M. E. Bailey and D. A. Williams. Cambridge University Press: 1988. Pp.573. £40, \$69.50.

IN 1784 William Herschel discovered that there were 'holes in the sky', and these black, star-less regions were photographed by E. E. Barnard around the turn of this century. Coupled with the discovery of a general reddening of the light from distant stars, Herschel and Barnard's observations were taken to indicate the presence of small, solid particles pervading the Galaxy. Until a few years ago this dust cloud was regarded as an annoyance that hindered and prevented optical observations of distant regions. Nowadays it is recognized as being an interesting phenomenon in its own right, giving us the chance to study particles of matter that are merely hundreds of microns across and that have existed for aeons in an ultra-high vacuum and at low temperatures. The dust is on the boundary between large-molecule chemistry and small-particle physics.

Recently there have been enormous advances in our ability to detect the infrared radiation from astronomical objects. The successful Infrared Astronomical Satellite (IRAS), new large ground-based infrared telescopes, sensitive CCD detectors and advanced laboratory work have enabled us to put together a comprehensive picture of the dusty regions in the galaxies. So the time was right for a large conference on the subject. The deliberations of just such a conference, held at the University of Manchester in December 1987, are recorded in the book under

review. The 55 papers convey the impression of an extremely lively area of research, and one in which there often seems to be little consensus. Many important and fundamental questions are still in need of an answer.

About one per cent of the mass of our Galaxy is in the form of dust. But is the dust content in equilibrium? Most of it (75 per cent) is formed in the expanding atmospheres of cool M giant stars by the nucleation of refractory atoms, followed by conglomeration. Supernovae, novae, planetary nebulae and C stars add the rest. The size and population is limited by thermal sputtering, sputtering caused by the impacts of energetic ionized molecules and simple grain-grain collisions. Shock waves from supernovae play a large part in these processes. All in all, a few solar masses of dust are being produced and destroyed each year.

The composition of the dust is another problem that occupies many of the contributors. Spectroscopy provides a host of clues. The 2175 Å ultraviolet 'bump' hints at small particles of graphite. The 9.7-µm infrared feature indicates silicates. Features at 3.3, 6.2, 7.7, 8.6 and 11.3 µm

are 'unidentified', and researchers variously suggest that they are due to polycyclic aromatic hydrocarbons (that is, dust smelling like coal-tar soap), hydrogenated amorphous carbon and silicate cores with accreted mantles of ice. The spectral features exhibit polarization, indicating that the dust is elongated and aligned. One idea is that particles might contain tiny superparamagnetic grains of magnetite and are thus lined up by galactic magnetic fields.

In the book, much is made of the fact that the dust was certainly present in the nebular cloud that condensed to form our Solar System. So evidence of the dust should be present in meteorites, comets and the zodiacal cloud.

The high quality of the papers in this volume, many of which are invited contributions by world experts, mean that it will be an essential reference work for many astronomers. *Dust in the Universe* is a very good book which deals with a subject where books — let alone good ones — are few and far between. □

David W. Hughes is in the Department of Physics, University of Sheffield, Sheffield S3 7RH, UK.

Birth pangs

Joseph Silk

The Early Universe: Facts and Fiction. By G. Börner. Springer-Verlag: 1988. Pp.439. DM 125, £41, \$69.

IN RECENT years, cosmology has become a respectable field. Five decades ago, many of the writings in this area were characterized by their mix of relativity and philosophy; now the topic is one in which forefront areas in particle physics and observational astronomy are brought together.

The Early Universe is one of the first textbooks to attempt a synthesis of the field from a modern perspective, and Börner is to be congratulated on his brave attempt to introduce the new cosmology to an audience at the level of a first-year graduate student in astrophysics. He tells a gripping story, although the writing is somewhat uneven and the background required of the reader is variable. Feynman diagrams and galaxy rotation curves, Higgs-field lagrangians and Tully-Fisher relations, make odd bedfellows. A kinder, gentler approach to the particle physics theory might have helped overcome the interdisciplinary barriers.

Nonetheless, the book merits close attention. The first four chapters introduce the standard cosmological models and provide a concise, topical review of observational cosmology. We learn about the fundamental facts of cosmology, including determinations of the age of the Universe

and of its mean density. Cosmologists have been striving for many years to reconcile facts and theory. Things haven't changed. We still have an age for the oldest globular cluster stars that exceeds, if the Universe is at critical density, the expansion age inferred to be two-thirds of the Hubble time, H_0^{-1} . To amend the situation, we can introduce a cosmological constant, as thoroughly described by Börner, or if we sympathize with Einstein's view that the cosmological constant was his greatest mistake, look elsewhere for the resolution. One can always criticize the observational age determinations, which present a tempting target, although any hitherto unrecognized sources of systematic bias towards lower globular cluster ages are in short supply.

The main loophole, however, is the premise that the Universe should be at critical density. That premise is based entirely on theoretical and even philosophical prejudice, epitomized by Ernest Barnes, the cosmologically inclined Bishop of Birmingham, who contended that "infinite space is simply a scandal to human thought". All astronomical observations point to an open Universe, destined to expand forever, whose age is H_0^{-1} , which for the acceptable value $H \approx 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ one estimates to be about 15×10^9 yr. Such an age is perfectly compatible with all other age determinations.

Another pedestal of the standard model of cosmology, the hot Big Bang, is the cosmic microwave background radiation. The search for possible distortions from a black body spectrum and for deviations

New journals review

THIS year *Nature's* annual new journals review supplement will appear in the issue of 28 September. Publishers and learned societies are invited to submit journals for review, taking note that journals which first appeared after June 1987, and which issued at least four separate numbers by the end of April 1989, will be considered.

Those submitting journals for review should promptly send at least four different issues (the first, the most recent and any two others) of each title to: Tim Lincoln, *Nature*, 4 Little Essex Street, London WC2R 3LF, England.

For further information please see *Nature* of 4 May (p.24) or telephone Tim Lincoln on 01-836-6633 (011-44-1-836-6633 from the United States) extension 2414.

from isotropy continues vigorously at present, and one anticipates that such blemishes are inevitable in our observed Universe, given the degree of large-scale structure and galaxy formation activity. Nevertheless, the physicist's search for perfection can be indulged by retrodicting the observed Universe back to the first microseconds after the Big Bang. Then the energies reached rival those attainable in the largest particle accelerators, and we can begin to probe the fundamental nature of matter. At still earlier instants, we can study interactions that require vastly higher energies to be initiated. The ultimate symmetry between the fundamental forces is attainable when electromagnetic, weak and strong nuclear forces merge at similar strength at a temperature of roughly 10^{28} K.

The second part of *The Early Universe* provides a detailed exposition of the particle physics underlying the standard cosmological model. Börner delves into supersymmetry and grand unification schemes, affectionately labelled SUSY GUTS, and discusses exotic topics such as strings and superstrings. There are three principal legacies from these very early epochs. Relic particles provide a potential source of dark matter. The breaking of grand unification can lead to the observed baryon asymmetry of the Universe, as well as generation of an inflationary epoch of expansion. The inflationary period is characterized by an exponential expansion rate during the transition period of symmetry breaking, which provides an effective energy source, and helps account for the observed uniformity and spatial flatness of the Universe.

Inflation also provides a source of density fluctuations, through amplified quantum noise, that is responsible for the large-scale structures we observe. Galaxies and galaxy clusters form by the process of gravitational instability, which requires the presence of initial seed fluctuations of a specific amplitude. The third section of the book provides a topical account of the theory of large-scale structure formation. Linear theory is described, and the reader is initiated into the mysteries of cold and hot dark matter. Numerical simulations provide the final gloss to galaxy formation theory. One has to be constantly aware that the final outcome, no matter how closely it may resemble the observed Universe, is no more believable than the initial conditions that went into the initial value problem that we colourfully describe as the Big Bang. Until we find a theory of quantum cosmology that specifies the initial amplitude and spectrum of the primordial density fluctuations, we should remain reasonably sceptical about schemes for cosmic evolution. □

Joseph Silk is a Professor in the Department of Astronomy, University of California, Berkeley, California 94720, USA.

Time to turn to Marje?

R. N. Thompson

Igneous Petrogenesis. By M. Wilson. Unwin Hyman: 1989. Pp. 466. Hbk £50, \$100; pbk £24.95, \$39.95.

RESEARCH on the genesis of igneous rocks has produced a substantial flow of papers and a goodly crop of robust differences of opinion and interpretation. Both are hallmarks of active science. But for more than a decade nobody has settled down and made a really satisfactory attempt to distil the new material into a book, so that newcomers to the subject may find out what it is all about without drowning in a sea of undigested journal articles. Indeed, John Maddox was moved a while ago to make cross noises about part of the problem — the obscure private language of isotope geochemistry in *Nature*.

Marjorie Wilson has come to the rescue with a challenging book which looks at igneous petrogenesis from both physical and chemical viewpoints. Her own research field is predominantly geochemistry, and it is these aspects of her text which tend to be the more detailed and incisive. The first third of the book is a group of introductory chapters which set out the principles of the

subject clearly and include a satisfactory response to Maddox's complaint. The remainder deals with magmatism associated with the main terrestrial tectonic regimes, using the same format in each case: tectonic and geophysical setting, petrography, elemental and isotopic chemistry, petrogenesis. The result is extremely satisfactory because the reader can quickly flip from chapter to chapter in order to compare some specific aspect of magmas worldwide.

Nevertheless, it would be wrong to leave potential buyers thinking that they can throw away the rest of their libraries on this subject. Wilson has covered reams of controversial material within a book of reasonable length by simply ignoring all manner of hypotheses that are not to her taste. Her most spectacular omission is all mention of the predominant hypothesis (looking at the subject worldwide) for the genesis of granitic and associated rocks — the largest plutonic igneous rock group. This book should carry a health warning for any hypertensive geochemists who have spent their scientific lives considering that some, if not all, granites (*sensu lato*) originate by continental crustal melting during regional metamorphism. They will find that both their data and their ideas are absent. □

R. N. Thompson is a Professor in the Department of Geological Sciences, Science Laboratories, South Road, Durham DH1 3LE, UK.

JOINT LECTURE

(on the theme of the presentation of science through literature)

Translation or transformation?: the relations of literature and science

By Gillian Beer

(Reader in Literature and Narrative, and Professor Elect of English (1966) in the University of Cambridge)

Vigorous interchange of ideas and concerns between scientific and literary communities should not lead us to expect through-going and sustained congruity. Ideas rarely remain intact when they change generic context and their reception outside the immediate circle of co-workers may raise previously excluded questions. Changes of form as well as of semantics shape meaning. Scientific methods and ideas are often at their most powerful in literary works where it is possible to loosen the terminology and punningly transgress the terms provided. Tensions inhere in the very insistence on single signification in scientific discourse. Our analysis may founder if we seek a systematic representation of scientific theory within works of literature, or, indeed, a sufficient 'source' of scientific ideas within the common structure.

The lecture will examine, with examples, kinds of interchange between scientific and literary texts in the nineteenth-century and in our own time.

Tea will be served at 17.15.

Reproduced above is part of a document discovered on a university notice-board in London, England, circa April 1989. Analysis of the main text reveals the use of a hitherto unknown language, which some experts hold to be a daughter-tongue of English. Others differ. Readers are invited to decode the text, and provide a name for this curious language.

From spectrum to molecule

William Rhodes

Theory and Methods of Calculation of Molecular Spectra. By L. A. Gribov and W. J. Orville-Thomas. Wiley: 1988. Pp. 636. \$178, £86.

ONE of the central themes of molecular spectroscopy is the interplay between molecular structure and spectral structure, and how this interplay is governed by the relative motions of the electrons and nuclei in the molecule. Although the theoretical basis is well understood in terms of the Born–Oppenheimer scheme and its ramifications, it is surprising that there are few books which treat the subject in sufficient detail to provide a proper grounding for students entering the field. Any serious attempt to remedy the situation should therefore be appreciated. Gribov and Orville-Thomas's book is presumably such an attempt; it is aimed not only at the beginner, but also at those who wish to understand rather sophisticated methods of relating molecular and spectral structure.

The first five chapters give an elementary, but wide-ranging, treatment of the adiabatic (Born–Oppenheimer) approximation, the Franck–Condon principle, vibronic coupling, molecular–electronic structure, symmetry and related subjects. The following seven offer a detailed formulation of such specific topics as kinetic coefficients, the harmonic approximation, force constants and infrared spectra. Finally, there are three chapters which contain a symbolic logic approach to spectroscopy and methods of calculating electronic and Raman spectra. Throughout, there is an emphasis on developing spectroscopic quantities in terms of elementary molecular entities, such as atomic orbitals and internuclear geometric parameters. This makes for a lot of detailed algebra.

To be sure, Gribov and Orville-Thomas bring their own flair and flavour to bear on the subject. They are clearly experts in this field and have developed a perspective and methodology in a way that few (if any) others could do. Nevertheless, the book is not without shortcomings. In the preface, the authors say that their purpose is to show "how computers can be used to supplement, and in some cases to replace, experimental spectroscopic studies . . .". Yet, except for Chapter 13, which deals with symbolic logic, the book has little to do directly with computing. It does not even bring the reader to the brink of computing, although the stress throughout is on algebraic formulation of quantities and parameters which could easily be subjected to computational algorithms.

Furthermore, the authors do not seem to have a definite audience in mind. Many chapters blend introductory explanations and sophisticated, abstract formulations in a manner which seems to be pedagogically inconsistent. For example, in Section 3.1 great care is taken to explain the meaning of hermitian operators, yet elsewhere in the book many other operations and concepts that are less widely known are used with little explanation. The simplest concepts of symmetry are introduced pictorially in Section 5.1, yet this is followed by a sophisticated technique involving circulants rather than the usual group-theory approach. The point is that anyone who needs the pictorial introduction is quite likely to be bewildered by the algebra that follows.

Perhaps the most serious criticism applies to the first three chapters, which lay the theoretical foundations. Here, the authors seem more intent on justifying their practical approach to molecular spectroscopy, in a manner that is oblique and discursive, and even philosophical at times, rather than on making a clear exposition of the basics for the beginner. Considerable emphasis is placed on what

is called the "inverse spectral problem", a method of adapting theoretical models to experimental spectra, but one which will probably remain nebulous to the reader until Chapter 13, where it is applied. The early chapters also contain a number of pedagogical errors and poorly drawn figures. These include incorrect statements about the relative value of electronic and vibrational energies (p. 22), impossible curves for vibrational wavefunctions (Fig. 2.7), and an unnecessary obfuscation of the mirror symmetry law of absorption and emission spectra depicted in Fig. 2.8.

These weaknesses in the underlying fundamentals, together with the fact that calculational methods *per se* are not dealt with, suggest that perhaps a better title would have been *Theoretical Methods of Molecular Spectroscopy*. But as a book on the ways of relating spectral parameters to molecular parameters, it is quite strong and should be a valuable resource to anyone who wishes to go beyond the basics of the subject. □

William Rhodes is a Professor in the Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, Florida 32306, USA.

World of decay

Adrian Bath

Isotopes in the Earth Sciences. By Robert Bowen. Elsevier Applied Science: 1989. Pp. 647. £80, \$144.

STUDIES of natural isotope systems have had a vast influence on the Earth sciences. They are one of the most effective ways by which to quantify geosphere parameters — time, temperature, source identification and mixing — and have been a driving force for technical innovation in the sophisticated and expensive business of isotopic measurement. Robert Bowen addresses his book to a broad range of Earth and environmental scientists as a comprehensive guide through the scientific and technical complexities of the subject.

Since its early days, isotope geoscience has been dominated by its geochronological applications, in much the same way that the immediate relevance of carbon-14 dating has appealed to archaeologists. This historical dominance is reflected by half of the book dwelling on geological dating and its interpretative problems. To many it must seem that isotope studies create or define as many problems as they solve; they illustrate how complex and intractable Earth processes are.

The second half takes a lateral view of the roles of isotopes in our understanding of the spectrum of processes in geosphere–hydrosphere–biosphere systems. This is

the most exciting aspect of the subject, but treatment here of the assorted topics varies between excessive detail and undue superficiality, and never finds a satisfactory balance or unifying approach. The final chapter covers something entirely different — the historical and political background to nuclear power and radioactive waste disposal, presumably in recognition of their perceived link with all isotopes.

Bowen's book is essentially an encyclopaedic and uncritical catalogue of developments in isotope studies up to the present. Disappointingly little space is devoted to looking ahead. New research needs to be directed towards predictive Earth studies and towards understanding the difficult but challenging interfaces between the geosphere, hydrosphere and biosphere. The Earth scientist is unique in being concerned with processes over widely ranging timescales in the past. Prediction of the future is a logical but difficult progression, and our challenge is to seek out further ways in which isotopes will improve the scientific basis of such prediction. It hardly needs adding that, in an area of research which demands ever greater investment of resources, there is a need to educate an audience beyond the already-convinced Earth scientist. □

Adrian Bath is in the Fluid Processes Research Group, British Geological Survey, Keyworth, Nottinghamshire NG12 5GG, UK.

● The Uranium Institute, London, has published the proceedings of its most recent symposium under the title *Uranium and Nuclear Energy: 1988*. Price is £52, \$100.

Structure and function of bacterial photosynthetic reaction centres

G. Feher, J. P. Allen, M. Y. Okamura & D. C. Rees

The primary reaction of photosynthesis is light-driven charge separation, carried out in reaction centres, which are complexes of integral membrane proteins and cofactors. The recent determination of the crystal structures of the reaction centres of two photosynthetic bacteria provides a basis for a quantitative understanding of the primary electron transfer processes of photosynthesis.

PHOTOSYNTHESIS is the biological process by which electromagnetic energy (light) is converted into chemical energy. Life on Earth derives all its energy from this process. Two main classes of organisms perform photosynthesis: those that evolve oxygen, for example, green plants, and photosynthetic bacteria that do not. The process in bacteria is much simpler; for example, bacteria contain only one photosystem, whereas green plants contain two, photosystems I and II. A great deal of progress has been made in the past two decades in understanding the molecular structure and function of the simpler bacterial photosynthesis system, much of which is also applicable to the photosynthetic apparatus in green plants. Here we focus on the early (primary) events of bacterial photosynthesis.

The primary process of bacterial photosynthesis is a trans-membrane charge separation, schematically illustrated in Fig. 1. Absorption of a photon excites the primary electron donor, a pigment molecule, from its ground state D to its excited state D^* . This is followed by a transfer of an electron from D^* to the acceptor A_1 . The electron is subsequently shuttled through a series of acceptors, A_2 , A_3 and the missing electron on D^+ is replaced by a secondary donor (not shown in Fig. 1). The remarkable feature of this electron transfer chain is that the absorption of *one* photon results in the formation of *one* charge-separated pair, that is, the quantum yield of the process is close to its maximum value, unity—an achievement that remains unmatched in photochemical reactions in model systems.

The minimum structural unit capable of producing the charge separation is an integral membrane complex termed the reaction centre. Its existence was first postulated in 1932¹, and direct, spectral evidence for its presence in bacteria was obtained in 1952². It was only around 1970, however, that a complex capable of performing the charge separation was isolated from the purple photosynthetic bacterium *Rhodospirillum rubrum* (*Rb.*) *sphaeroides*³⁻⁵. In the past two decades, great strides have been made in under-

standing the structure and mode of action of this reaction centre (for reviews see refs 6 and 7). These advances have resulted from the convergence of several biochemical and biophysical techniques that have provided information about the functions of the reaction centres together with their static and dynamic structures. Recently the high-resolution ($<3\text{\AA}$) determinations by X-ray crystallography of the spatial arrangements of the various molecular donors and acceptors that comprise the reaction centres of the related species *Rhodospseudomonas* (*Rps.*) *viridis* and *Rb. sphaeroides*⁸⁻¹⁴ have paved the way for a quantitative analysis of the kinetics of the electron transfer reactions involved in the primary charge separation.

Reaction centre composition

The reaction centre is an integral membrane protein that contains three subunits, L, M and H, and the following cofactors: four bacteriochlorophylls, two bacteriopheophytins, two quinones and one non-haem high-spin Fe^{2+} (refs 6, 7). The primary reactants were identified largely by spectroscopic methods. In particular, as the products of the primary steps are unpaired electrons, electron paramagnetic resonance (EPR) and the related technique of electron-nuclear double resonance (ENDOR), as well as optical spectroscopy were used to delineate the reactants and initial sequence of events. Thus, by the middle of the 1970s, it was established that the primary donor is a bacteriochlorophyll dimer¹⁵⁻¹⁸, the primary and secondary acceptors are quinones¹⁹⁻²¹ and a transient, intermediate acceptor is a bacteriopheophytin²²⁻²⁴. The secondary donor in *Rb. sphaeroides* is an exogenous water soluble cytochrome c_2 , whereas in *Rps. viridis* it is a multihaem cytochrome which forms an integral part of the reaction centre.

Electron transfer functions and energetics

The reaction centre can be viewed as a protein scaffolding that holds the various cofactors (reactants) in their appropriate positions for efficient electron transfer. The kinetics of the individual electron transfer reactions, summarized in Fig. 2, were determined by time-resolved optical spectroscopy (reviewed in ref. 26). The high quantum yield discussed in connection with Fig. 1 is the result of each forward reaction being 2-3 orders of magnitude faster than the competing back (charge-recombination) reactions. The price paid for this high quantum yield is a significant loss in energy of about 60-70% in producing the final charge-separated state (Fig. 2). The main challenge is to understand quantitatively the rates at which electrons are transferred between cofactors. Because these rates depend in a sensitive way on the orientations and distances between cofactors and on the electron densities at the different atomic positions of the cofactors, to achieve this we must know both the spatial and the electronic structures of the reactants.

Three-dimensional structure

The method of choice for obtaining the three-dimensional structure of macromolecules is X-ray diffraction. This technique

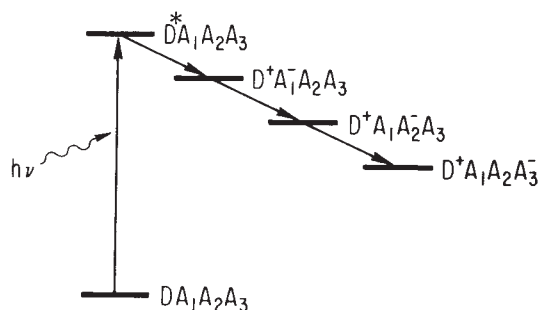


FIG. 1 Schematic representation of the initial charge separation in bacterial photosynthesis. The absorption of a photon is followed by ejection of an electron from the excited donor D^* . The electron is transferred to the acceptor A_1 and then shuttled through a series of acceptors A_2 , A_3 , ... This is shown in more detail in Fig. 2. The minimum unit capable of producing the charge separation is called the reaction centre.

requires relatively large (several tenths of a millimetre), well ordered single crystals. Until recently, it was generally believed that integral membrane proteins could not be crystallized because of the presence of randomly oriented detergent molecules that would interfere with the formation of an ordered array of identical units. This myth was shattered in 1980 by the successful crystallization of bacteriorhodopsin²⁷ and porin²⁸. The fallacy of the argument was that many of the points of contact of the macromolecules are hydrophilic and thus contain no detergent²⁹.

The first structure of a membrane protein to be solved at atomic resolution (2.9 Å; recently refined to 2.3 Å (ref. 30)) was that of the reaction centre from *Rps. viridis*^{8-10,31}, a signal achievement that was recognized by the award of the 1988 Nobel Prize for Chemistry to J. Deisenhofer, R. Huber and H. Michel. Soon thereafter, the reaction centre from *Rb. sphaeroides* was crystallized³²⁻³⁴ and its structure has now been determined to a resolution of 2.8 Å (refs 11-14, 35, 36). Preliminary phases of the X-ray diffraction and preliminary structures of reaction centres from *Rb. sphaeroides* were obtained by the molecular replacement method, using the coordinates of the reaction centre from *Rps. viridis*³⁷⁻³⁹.

As well as having crystals that diffract well, a knowledge of the primary sequence of the protein greatly assists the construction of the three-dimensional structure from electron density maps. The poor solubility of the reaction centre made it difficult to determine the amino-acid sequences by traditional methods but the amino-terminal sequences of the three subunits were obtained in the late 1970s⁴⁰, making it possible to sequence the reaction centre by recombinant DNA techniques⁴¹⁻⁴⁴.

The three-dimensional structures of the reaction centres from *Rb. sphaeroides* and *Rps. viridis* are similar; we present here the main structural features of both, emphasizing features conserved between the two. The main differences between them are that *Rps. viridis* has an additional cytochrome subunit containing four haem groups, bacteriochlorophyll b instead of bacteriochlorophyll a, and the primary quinone, Q_A , is menaquinone rather than ubiquinone.

The structure of the cofactors is shown in Fig. 3. The cofactors are arranged along two branches, A and B (termed L and M in *Rps. viridis*⁸⁻¹⁰), which are approximately related to each other by a two-fold symmetry axis extending from the iron to two closely associated bacteriochlorophylls (D_A and D_B) that form the dimer. After optimizing the rotation axis that relates equivalent cofactors in the two branches the root-mean-square

deviation between equivalent atoms is of the order of 1 Å (refs 11, 36).

Although the A and B branches are nearly symmetrical, the rate of electron transfer is at least a factor of 20 larger along the A branch (see ref. 45). This difference seems to be due to small deviations from the 2-fold symmetry of both the cofactor and the protein subunits (see below). The arrangement of the pigments along the A branch is consistent with the electron pathway as predicted from spectroscopic measurements (Fig. 2), although the existence of two symmetry-related branches had not been anticipated.

The bacteriochlorophylls D_A and D_B overlap at the ring I position to form the bacteriochlorophyll dimer that had been identified as the primary donor by spectroscopic techniques¹⁵⁻¹⁸. The distance between ring centres is 7.0 Å and the average distance between the planes of rings I in *Rps. viridis* is ~3.0 Å (ref. 8) and in *Rb. sphaeroides* ~3.5 Å (refs 11 and 13). Next along each branch are bacteriochlorophyll monomers, B_A and B_B , that are potentially involved in facilitating electron transfer from the donor to the bacteriopheophytin ϕ_A and ϕ_B . Optical studies had indicated that one of these is the intermediate acceptor²⁶ whereas the other is not involved in the electron transfer. The two quinones Q_A and Q_B are the primary and secondary acceptors. The identification of Q_A as the primary quinone is based on the X-ray structure of the *Rps. viridis* crystals, which are photochemically active; that is, they form $D^+ Q_A^-$ under illumination, but lack Q_B (ref. 8). The structure (Fig. 3) suggests that the active bacteriopheophytin is on the A-branch as it lies closer to the primary acceptor Q_A . The Fe^{2+} is located between the two quinones; it is coordinated to four histidines and to the bidentate ligand of a glutamic acid. These five residues are conserved between *Rps. viridis* and *Rb. sphaeroides*. The ligands form a distorted octahedron; the Fe^{2+} in *Rb. sphaeroides* is closer to Q_B than to Q_A by approximately 2 Å, as predicted from spectroscopic measurements⁴⁶.

The structure of the protein backbone of the reaction centre is shown in Fig. 4. In agreement with the predictions based on the distribution of hydrophobic amino-acid residues in the sequence of each subunit⁴¹⁻⁴⁴, the results of X-ray diffraction indicate the presence of 11 transmembrane helices, five each for the L and M subunits and one for the H-subunit. The L and M chains are in close contact; the LM complex forms a cylindrical core with an elliptical cross section with axes ~40 Å and 70 Å. This core region contains a bundle of four α helices, two from each subunit¹², and each helix contains a histidine residue which

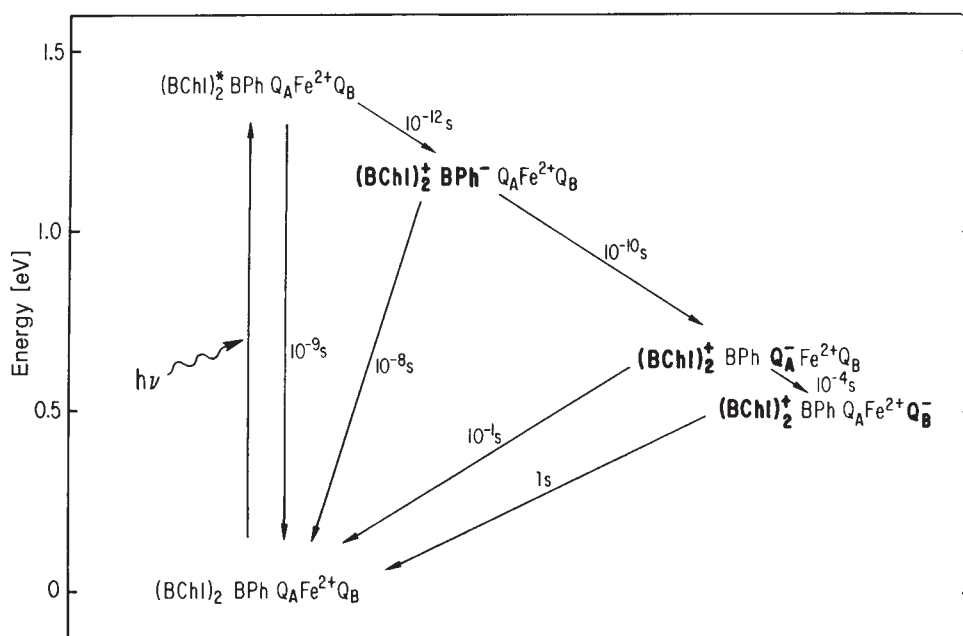


FIG. 2 Electron-transfer reactions in reaction centres of photosynthetic bacteria. After photon absorption, the electron transfers through a series of reactants that are stabilized against charge recombination for progressively longer periods. Charged donor-acceptor species are shown in bold face. Transfer times are given for room temperature and are approximated to the nearest power of 10. Energies are approximately to scale. The ratios of forward rates (arrows pointing right) to charge-recombination rates (arrows pointing left) are 10^2 - 10^3 . These large ratios are responsible for the high quantum yield. (BChl)₂ is a bacteriochlorophyll dimer which is the primary donor; (BChl) is a bacteriochlorophyll monomer; BPh bacteriopheophytin, which is the intermediate acceptor; Q_A and Q_B are ubiquinones, which are the primary and secondary acceptors (subscripts refer to the two branches A and B). Fe is a high-spin ferrous iron. Logically, the bacteriopheophytin (BPh) should be called the primary acceptor. However, its role was discovered only after the other acceptors; it was, therefore, named the 'intermediate' or 'transient' acceptor. The electron pathway of the charge recombination of $D^+ Q_B^-$ proceeds via the thermally excited state $D^+ Q_A^-$. The direct recombination time of $D^+ Q_B^-$ is at least an order of magnitude slower than the observed time of 1 s (ref. 25). $D_A D_B = (BChl)_2$, B = BChl, ϕ = BPh, C = carotenoid. Electron transfer proceeds preferentially along the A branch.

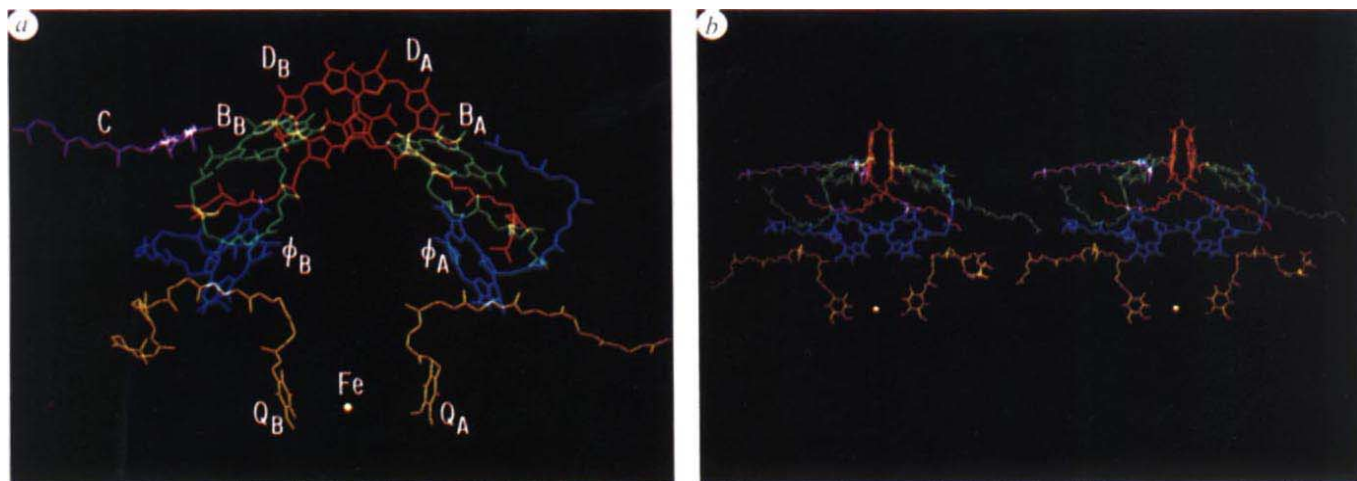


FIG. 3 Cofactor structure of the reaction centre from *Rb. sphaeroides* (wild type strain 2.4.1). *a*, The symmetry axis is aligned vertically in the plane of the paper. *b*, A stereo reconstruction rotated from the view in *a* by a 90° clockwise rotation around the two-fold symmetry axis with the direction

defined from the Fe to the dimer. (Modified from ref. 11). Subscripts refer to the two branches A and B; electron transfer proceeds preferentially along the A branch [$D_A D_B = (BChl)_2$, $B = BChl$; $\phi = Bph$, $C = \text{carotenoid}$].

FIG. 4 Stereo view of the structure of the reaction centre from *Rb. sphaeroides* 2.4.1. with the L (yellow), M (blue) and H (green) subunits, the carotenoid (purple) and the cofactors (red). The top (periplasmic side) of the reaction centre is the docking site for cytochrome c_2 . In *Rps. viridis* a four-haem cytochrome forms an integral part of the reaction centre on the periplasmic side. (Modified from ref. 12.)

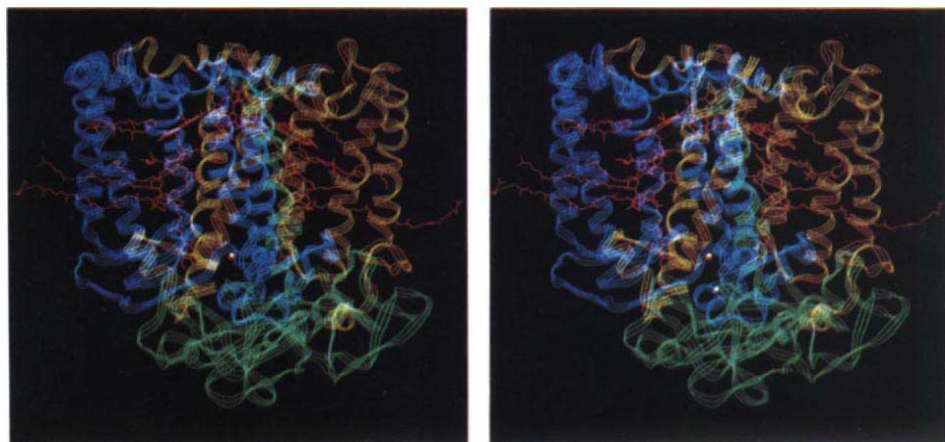
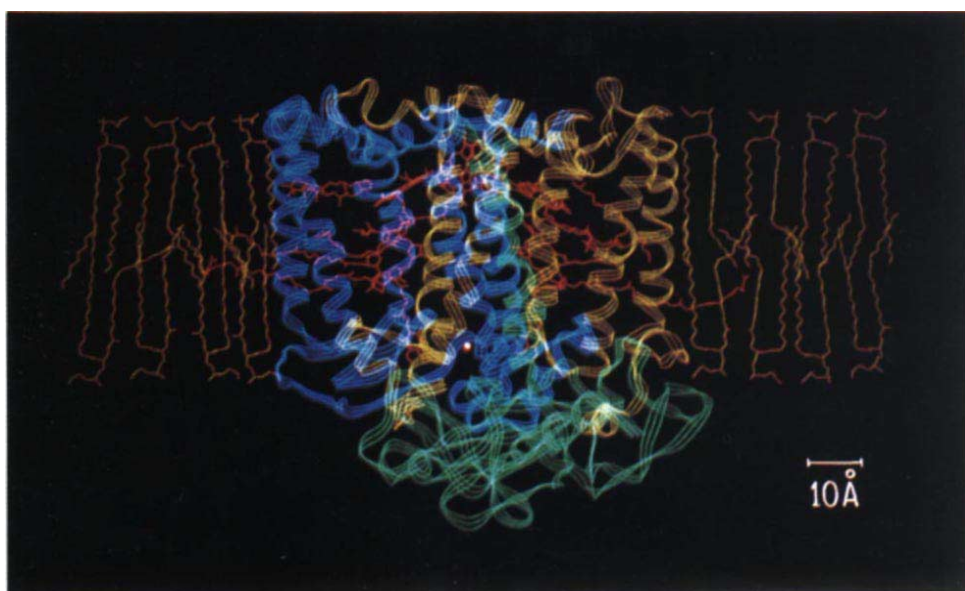


FIG. 5 The position of the reaction centre from *Rb. sphaeroides* 2.4.1 in the lipid bilayer. For simplicity, only one major type of lipid, phosphatidylethanolamine, is shown. Its disorder in the membrane is represented schematically. The two-fold symmetry axis is in the plane of the paper joining the Fe (dot) near the cytoplasmic side (bottom) with the bacteriochlorophyll dimer near the periplasmic side (top). Colour code as Fig. 4. (Modified from ref. 35.)



ligands to the iron, suggesting that the metal ion plays a part in stabilizing the core region. All the cofactors are associated with the LM complex, as was postulated⁶.

The LM complex also has a two-fold rotation symmetry axis that approximately relates the two subunits. The symmetry axes of the cofactors and the LM subunits are essentially equivalent. The similarity of the two subunits is most dramatically demonstrated by superimposing the M subunit on to the L subunit by a 180° rotation in which case the root-mean-square deviation between equivalent C α atoms of the five transmembrane helices is ~1.3 Å (ref. 36). The H chain forms a distinct globular unit that makes extensive contact with the cytoplasmic side of the L and M subunits. These contacts may stabilize the reaction centre structure. When the H subunit is removed, electron transfer from Q $_A^-$ to Q $_B$ is impaired⁴⁷: this may be due to a loosening of the structure of the LM complex which lowers the binding constant of Q $_B$. The periplasmic (top) side of the reaction centre from *Rb. sphaeroides* is the docking site of cytochrome c_2 , which serves as the secondary donor, that is, it delivers an electron to the oxidized bacteriochlorophyll dimer. In *Rps. viridis*, a cytochrome located on the periplasmic side of the LM complex forms an integral part of the reaction centre⁸.

The location of the reaction centre with respect to the membrane (Fig. 5) could not be directly determined by X-ray diffraction, because most detergent molecules surrounding the reaction centre are disordered and do not therefore contribute to the observed electron density. Qualitatively the central region of the reaction centre, which is devoid of charged residues is presumed to be inside the membrane. To determine more rigorously the association of the reaction centre with the membrane, we calculated the energy of interaction of the surface atoms of the reaction centre with the hydrophobic membrane lipids and the hydrophilic outside solvent using the energy function of Eisenberg and McLachlan⁴⁸. By systematically varying the translation and orientation of the reaction centre, its position in the membrane with a minimum energy was determined. The symmetry axis was found to be perpendicular to the plane of the membrane and the position within the reaction centre of a 40-Å-wide hydrophobic region, in contact with the lipid of the plasma membrane, was identified. The deduced position of the reaction centre in the lipid bilayer is shown in Fig. 5. The structure obtained by X-ray diffraction confirms the main features that had been deduced prior to crystallization (see for instance Fig. 35 of ref. 49).

Electronic structure of cofactors

Several optical and magnetic resonance techniques have been used to explore the electronic structure of the reaction centre, particularly electron-nuclear double resonance (ENDOR), which provides information about the unpaired *valence* electron that participates in the primary photochemical reactions. This information is not available from X-ray crystallography, which is based on the diffraction of the *core* electrons. More specifically, ENDOR measures the strength of the magnetic interaction between the valence electron spin and the surrounding nuclear spins, thus yielding a map of the spin density and the electronic wavefunction of the unpaired electron.

ENDOR experiments have been performed on the primary donor D $^+$ (refs 17, 18, 50), the intermediate acceptor ϕ_A^- (ref. 51) and on the acceptors Q $_A^-$ and Q $_B^-$ (ref. 52). The electron densities derived from these experiments have been used to check the accuracy of molecular orbital calculations of the cofactors (reviewed in ref. 53) and to calculate electron transfer rates⁵⁴ (see next section). ENDOR can also be used to determine features of the three-dimensional structure, such as hydrogen bonding that is not available from X-ray diffraction. For example, the two carbonyl oxygens of the quinones were shown to be hydrogen bonded and the O...H distances were determined to an accuracy of ~0.1 Å (ref. 52).

Optical⁵⁵, infrared⁵⁶ and Raman⁵⁷ spectroscopy have also

been used to investigate electronic structures. Of particular interest to electron transfer theories is the effect of an electric field on the optical spectra (Stark effect)⁵⁸⁻⁶¹. It was found that the Stark effect of the infrared band of the bacteriochlorophyll dimer (at 865 nm) is several times larger than that of the other bands, which is consistent with the presence of an excited charge transfer state that may be involved in the initial electron transfer.

Structure-electron transfer relationship

The spatial arrangement of the cofactors (Fig. 3) is crucial for the rates and specificities of the electron transfer reactions. According to the theory of Marcus⁶², the direct electron transfer rate between two molecules depends on three factors: the overlap of the electron densities (wavefunctions) of the two molecules; the difference in redox potential of the molecules; and a quantity called the reorganization energy, which is related to the energy associated with the rearrangement of atoms around the molecule after it loses or gains an electron. An example of the latter is the reorientation of solvent dipoles in the electric field created by the electron charge. The theory predicts that the maximum electron transfer rate (for a given overlap) occurs when the reorganization energy equals the difference in redox energy and that, under these conditions, the process is temperature independent. Several of the electron transfer reactions in reaction centres satisfy this condition.

The structure, both spatial and electronic, is essential to the calculation of electron density overlaps; a change in the distance between cofactors by a fraction of an ångström can change the electron transfer rate by orders of magnitude. The redox potentials are usually determined experimentally. The reorganization energy is often difficult to calculate accurately, but it is not as critical as the overlap in determining the transfer rate.

An important feature of the electron transfer reactions is the preferential direction of electron transfer along the A-branch. This arises from the breaking of the two-fold symmetry at several places in the structure of the reaction centre. First, there are inherent asymmetries in the dimer structure, for example, one of the tetrapyrrole rings is less planar (more puckered) than the other; furthermore, in *Rb. sphaeroides*, the magnesium coordination of the two halves is different. Second, the overlap of the dimer with B $_A$ is larger than with B $_B$. Third, the bacteriopheophytin ϕ_A is ~1.5 Å closer to B $_A$ than ϕ_B is to B $_B$. Fourth, the detailed binding of the cofactors to the protein backbone is different in the two branches. Fifth, the distribution of charged amino acids is asymmetrical. Lastly, the phytyl and isoprenoid chains do not obey the two-fold symmetry. The effect on the electron transfer rate of some of these structural asymmetries has been calculated for the reaction centres from *Rps. viridis*^{45,54} to give a ratio of the rates along the A and B branches of 45, which is consistent with the experimentally determined limit of >20 (ref. 45).

The primary electron transfer D $^+\phi_A \rightarrow D^+ \phi_A^-$ occurs in ~3 ps (for a review see ref. 26). It is difficult to account for this short transfer time given the relatively large distance of 17 Å between the centres of the tetrapyrrole rings of D and ϕ_A and the correspondingly small overlap of the electron densities. Consequently, the bridging monomer, B $_A$, has been invoked to account for the observed transfer rate⁶³⁻⁶⁶. In the simplest picture, a two-step sequential mechanism is proposed, in which B $_A^-$ serves as a transient, intermediate state, but this state was not observed using femtosecond spectroscopy. This can be explained by assuming that the D $^+B_A\phi_A \rightarrow D^+B_A\phi_A^-$ step is much faster than 3 ps, resulting in an undetectable population of B $_A^-$. An alternative mechanism, 'superexchange', has been postulated, in which the electronic overlap between D $^+$ and ϕ_A is effectively enhanced by a virtual state D $^+B_A^-$ (refs 64, 66). In addition to B $_A$, other bridging molecules may play a role in electron transfer: for example, there is a tyrosine at M210 in *Rb. sphaeroides* and M208 in *Rps. viridis* between D and ϕ_A and the phytyl chain of D $_A$, which makes van der Waals contacts

with ϕ_A . Thus, the detailed mechanism of this important first step remains to be elucidated.

The electron transfer from ϕ_A^- to Q_A is independent of temperature with a characteristic time of ~ 200 ps for both *Rb. sphaeroides* and *Rps. viridis*²⁶. The aromatic ring of the tryptophan at M252 in *Rb. sphaeroides* and M250 in *Rps. viridis* is approximately parallel to the quinone ring and is in van der Waals contact with both ϕ_A and Q_A ; it is, therefore, likely to be important in the electron transfer process. Plato *et al.*⁶⁷ conclude from theoretical calculations based on the three-dimensional structure that the tryptophan serves as a bridging (superexchange) mediator in the reduction of Q_A . Substitution of tryptophan with valine in the reaction centre from *Rhodobacter capsulatus* by site-specific mutagenesis drastically reduces photosynthetic growth⁶⁸. As neither the three-dimensional structure of the reaction centre of this mutant nor its electron transfer rate from ϕ_A^- to Q_A has been reported, the structural and kinetic basis of this effect remains to be established.

The electron transfer from Q_A^- to Q_B occurs in ~ 100 μ s (reviewed in ref. 69). As both quinones in *Rb. sphaeroides* are ubiquinone, the vectorial electron transfer from Q_A^- to Q_B must result from the difference in the environments of the quinones, which lowers the energy of Q_B^- relative to Q_A^- . Two structural differences can contribute to the lowering of the energy of Q_B^- (ref. 14): there are more polar residues in the vicinity of Q_B than Q_A , which would lower the energy of Q_B^- through dipole interactions; and the Fe^{2+} is closer to Q_B than to Q_A . As a consequence the electrostatic potential due to the positive charge of the metal ion lowers the energy of Q_B^- relative to Q_A^- . The Fe^{2+} in *Rps. viridis* is more symmetrically located between Q_A and Q_B ; in this system, the vectorial electron transfer from Q_A^- to Q_B could be accounted for by the inherent difference between the redox potential of menaquinone (Q_A) and ubiquinone (Q_B).

The rate of electron transfer from Q_A^- to Q_B has an activation energy of 560 meV (ref. 70). This large activation energy can be explained by the presence of polar residues in the Q_B binding site. According to Marcus' theory⁶², reorientation of the dipoles associated with these residues in the vicinity of Q_B^- will give rise to a reorganization energy that is much larger than the free energy difference between $Q_A^-Q_B$ and $Q_AQ_B^-$, leading to a relatively high activation energy.

The absence of a change in the electrical potential across the membrane associated with the charge transfer from Q_A^- to Q_B (refs 71, 72) is consistent with the structure, which shows that the line joining the centres of the quinones is approximately parallel to the surface of the membrane. By contrast, but also

in accord with the structure, the electron transfers from D^* to ϕ and from ϕ^- to Q_A are accompanied by changes in potential across the membrane.

The direct charge recombination rate of Q_B^- with D^+ is at least two orders of magnitude smaller than that of Q_A^- with D^+ (ref. 25), although the two quinones are approximately equidistant from the dimer (22 Å edge-to-edge). The most likely explanation of this difference is that the direct recombination of Q_B^- with D^+ is a thermally activated process whereas that of Q_A^- is independent of temperature. Although the difference is believed to be due to the presence of polar residues in the Q_B binding site, an alternative explanation is that the recombination proceeds through the protein along a different pathway.

Why are the forward electron transfer rates much greater than the recombination rates? This important question relates to the unity quantum yield; several explanations for this phenomenon have been proposed. (1) The forward reaction is aided by judiciously positioned intermediates, such as cofactors or amino-acid residues. (2) The molecular orbital overlap between a pair of states involved in the forward reaction is more favourable than that of the charge separated states; this becomes more pronounced as the distance between pairs of charged states becomes progressively larger as the electron proceeds towards Q_B . (3) A conformational change accompanies the charge separation making the recombination less favourable. (4) The large energy difference between a charge-separated and ground state, compared with the energy difference between two successive electron transfer states, can under certain conditions (when, for example, the reorganization energy is small) result in a reduced rate⁶². Relatively little work has been done on calculating the charge recombination rates, so the quantitation of the large ratio of forward to backward rates remains an open problem.

Subsequent electron transfer steps followed by protonation of Q_B^{2-} . So far we have discussed only the kinetics of the charge separated species resulting from the transfer of the first electron. However, the electrostatic energy associated with the final charge-separated species $(BChl)_2^+ BPh Q_A Fe^{2+} Q_B^-$, shown in Fig. 2, cannot be directly converted into chemical energy, that is, into ATP. According to the chemiosmosis theory⁷³, a proton gradient across the membrane is required, which can be formed only after a second electron reduces Q_B^- to Q_B^{2-} . The doubly reduced quinone picks up two protons to form QH_2 , which leaves the reaction centre (shaded circle in Fig. 6) to be replaced by an exogenous neutral quinone (reviewed in ref. 74). This is a cyclic process which requires the presence of exogenous quinones and cytochrome c_2 (Fig. 6). Before a second photon can be absorbed, D^+ must be reduced, a task accomplished by cytochrome c_2^{2+} (lower right quadrant in Fig. 6). A typical cycling time is 10^{-3} s.

The main problem associated with the protonation of Q_B^{2-} concerns the mechanism by which protons transfer to the doubly reduced quinone. In one proposed model, protons are transferred from the outside solvent to Q_B^{2-} through a chain of protonatable residues—a process analogous to a 'bucket brigade'. The reaction centre structure shows two chains of residues that could form proton bridges from Q_B^{2-} to the outside¹⁴. To investigate this mechanism, the protonatable residue closest to Q_B , the glutamate at L212, was changed by site-specific mutagenesis to glutamine⁷⁶. The cycling rate (Fig. 6) in this mutant was reduced by more than an order of magnitude, which provides strong evidence for the involvement of Glu L212 in the protonation of Q_B^{2-} . It should be possible to map the entire protonation path by this technique.

Outlook

Now that the three-dimensional structure of the reaction centre has been determined and progress has been made in understanding the electronic structure of the cofactors, the stage is set for the quantitative understanding of the various electron transfer steps, and to this end some progress has already been made. But the mechanism of the initial electron transfer step, that is,

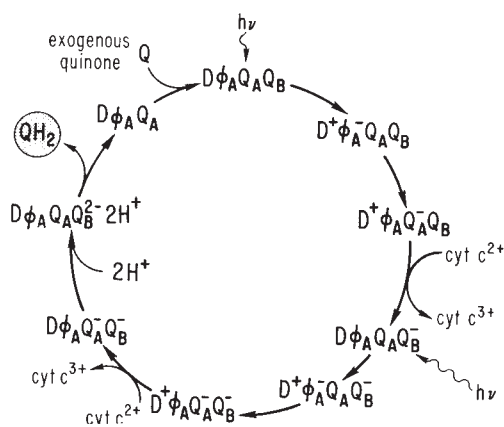


FIG. 6 Cyclic electron transfer through the reaction centre in the presence of exogenous quinone and ferrocyanide ($cyt\ c^{2+}$). The first few steps of the cycle (upper right quadrant) correspond to the transfer of the first electron shown in Fig. 2 [$D = (BChl)_2$, $\phi = BPh$]. A second electron produces the doubly reduced species Q_B^{2-} , which picks up two protons and leaves the reaction centre (shaded circle). Another membrane protein, the cytochrome bc_1 complex (not discussed here), is involved in shuttling the protons across the membrane and reducing ferrocyanide ($cyt\ c^{3+}$) (ref. 75).

whether it is superexchange or a two-step process, remains to be determined. It has been suggested on theoretical grounds that the effect of an applied electric field on the kinetics may decide between the two alternatives⁷⁷; preliminary experiments along these lines seem to favour superexchange⁷⁸. Another largely unanswered question concerns the role of the intervening amino acids, and phytol and isoprenoid chains in electron transfer. This question is being addressed both theoretically⁷⁹ and experimentally by site-specific mutagenesis⁶⁸, but the three-dimensional structures of the mutants need to be determined before a direct effect of an altered residue can be distinguished from an indirect effect resulting from a structural change.

Because the calculated electron transfer kinetics are so sensitive to the details of the structure, it is imperative to improve the accuracy of determination of the coordinates. Furthermore, the X-ray structure has so far given only a static picture of the neutral or ground state of the reaction centre. There is experimental evidence that structural changes occur during charge separation^{25,26} which, in principle, could be investigated with synchrotron radiation by pulsed X-ray diffraction techniques⁸⁰. The dynamic aspects of the reaction centre structure have been investigated theoretically by molecular dynamics calculations^{66,81}. Experimentally, information about the dynamical aspects could be obtained from the temperature dependence of the structural parameters, but no such experiments have been reported for reaction centres.

On a broad scale, our understanding of the primary processes in bacterial photosynthesis opens up exciting prospects for

studies of green plant photosynthesis, energy sources and membrane proteins. Comparing the bacterial system with the more complex photosystem II in green plants shows similarities between the cofactors as well as the L and M subunits of the bacterial reaction centre and the D₁ and D₂ subunits of photosystem II (refs 36, 41–44, 82, 83). In particular, a number of residues that form ligands to the cofactors, as well as certain residues that stabilize the protein, are highly conserved. The structural similarity postulated between the reaction centres of bacteria and photosystem II has already been used for targeting residues for site-specific mutagenesis (see ref. 84). As an energy source, the reaction centre is not the best possible compromise between quantum yield (approximately unity) and energy-conversion efficiency (~30%). A quantitative understanding of the electron transfer processes should make it possible to modify the RC and to design model systems that in some respect may be superior to the native RC.

As the only integral membrane protein whose three-dimensional structure has been determined at atomic resolution, the reaction centre also provides a model for understanding properties of membrane proteins such as folding⁸⁵. How valid these generalizations are will be known only when other membrane proteins, such as receptors, channels and transport proteins, have been crystallized and their structure determined. □

G. Feher, J. P. Allen and M. Y. Okamura are in the Department of Physics, University of California, San Diego, La Jolla, California 92093. D. C. Rees is in the Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024, USA.

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Properties, detectability and origin of interstellar diamonds in meteorites

Roy S. Lewis*, Edward Anders* & Bruce T. Draine†

* Enrico Fermi Institute and Department of Chemistry, University of Chicago, Chicago, Illinois 60637-1433, USA

† Princeton University Observatory, Princeton, New Jersey 08544-1001, USA

Laboratory studies suggest that microdiamonds will be quite elusive in interstellar space, being detectable only if >10% of the carbon is present as diamond. The diamonds apparently formed by condensation in cool stellar atmospheres, and were then impregnated with isotopically anomalous noble gases, such as Xe, from a supernova.

INTERSTELLAR diamonds were predicted to exist in 1969¹, but were discovered only in 1987, and then in meteorites² rather than in interstellar space. We have determined some of the properties of such diamonds, with the aim of elucidating their origin and to see how readily they might be detected in interstellar space.

These 'microdiamonds' (also called C δ) have a median size of only 26 Å, ten to one thousand times smaller than common interstellar grains. Their size distribution is log-normal³ rather than power-law, reflecting growth rather than fragmentation and suggesting a short interstellar residence time. They readily form colloids and behave as a polybasic weak acid (pK range 6–9; equivalent weight $\approx$ 1,700), apparently because of the presence of –COOH groups on the surface. The infrared spectrum shows absorption bands attributable to –CH, –COOH, C–O and N, but these bands, as well as the ultraviolet absorption edge of diamond, would be detectable in interstellar extinction only if more than 10% of the interstellar C were in the form of diamond. Several lines of evidence suggest that the microdiamonds formed metastably by circumstellar condensation^{2,4}, not by shock transformation of graphite grains⁵.

Diamond samples were isolated from the Murchison C2 and Allende C3V chondrites. The meteorites were dissolved in HCl–HF, non-diamond carbon was destroyed with hot HClO₄, and diamonds were extracted as a colloid in basic solution⁶. Sample purity was ascertained most thoroughly for Allende DM, which contained <1% elements of atomic number $Z > 6$, with 600 p.p.m. SiC being the principal impurity^{7,8}. On the basis of infrared spectroscopy, scanning electron microscopy–energy-dispersive X-ray analysis and noble-gas and carbon isotope analyses, the Murchison GL1 sample seems to have been somewhat less pure.

From the data available it seems that the diamonds from C2 and C3 chondrites are essentially similar⁷, except that the latter are somewhat depleted in nitrogen and lack two noble-gas components of normal isotopic composition, Ne-A1 and Xe-P3 which are thermally labile (release temperature $\leq$ 800 °C) and may have been lost during thermal metamorphism of C3 chondrites⁹.

Size distribution

The size distribution of Murchison GL1 was measured on a transmission electron microscope (TEM) at a magnification of 330,000 (ref. 3). Photographs were taken of areas thin enough for dark-field imaging to resolve single diamond crystals. The particles were irregular in shape, with ragged edges. The images were digitized and analysed by a computer program that recorded and measured particles whenever the rise in image bright-

ness at their edges exceeded a certain threshold value. The particle outlines drawn by the program generally agreed with those on the original photographs. From the area of each particle an effective circular diameter D_{eff} , was calculated. A possible source of error is overlap of particles, which would skew the distribution to larger sizes; but the particles imaged covered less than 10% of the field, so that such overlap should be slight.

The data for all 425 measured particles give an excellent fit to a log-normal distribution, with deviations exceeding the statistical error only for the first and last 2% (Fig. 1). The median diameter of 26 Å corresponds to only 1,060 atoms. This value is quite robust, changing only to 30 or 27 Å when the threshold is raised to eliminate those 30–33% of the particles that were faint (absolutely or relative to the local background). For spherical particles, the size distribution in Fig. 1 corresponds to a mass distribution with a median at 50 Å.

The form of the distribution is surprising, as interstellar dust normally shows a power-law distribution¹⁰, which is the steady-state form for fragmentation. A log-normal distribution reflects either size-sorting or growth followed by partial conversion of small grains to large ones¹¹, a spontaneous process (called Ostwald ripening) that lowers the surface energy.

The good fit down to small sizes suggests minimal contributions from processes that affect small grains preferentially, such as fragmentation, sputtering and erosion, and also from particle overlap in the TEM images. Unless these effects somehow cancel each other, it seems that the distribution is young and unevolved.

Acidic diamonds

A curious property of the microdiamonds, observed long before their identification, is their tendency to form colloids at high pH values, which coagulate at lower pH (refs 6, 12). A possible explanation is that weakly acidic groups such as –COOH are

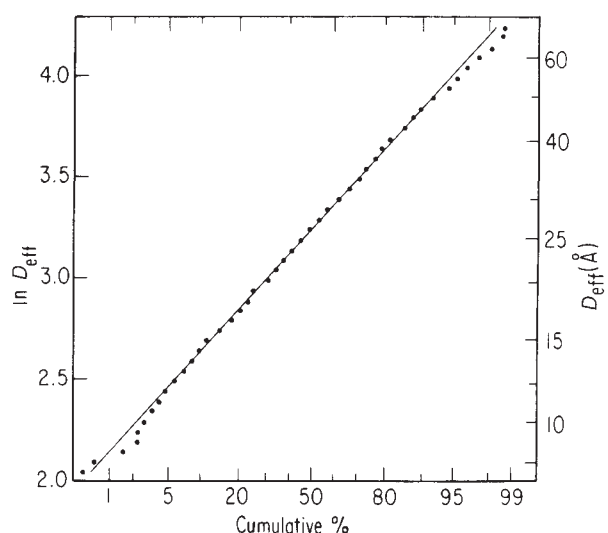
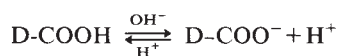


FIG. 1 Size distribution of the C δ microdiamonds in the Murchison GL1 sample³. The distribution is log-normal: $dP/d\ln a = (2\pi\sigma^2)^{-1/2} \times \exp[-(\ln(a/a_0)^2/2\sigma^2)]$, with $\sigma = 0.47$ and $2a_0 = D_{\text{eff}} = 26$ Å.

present on the surface of the microdiamonds, causing them to become negatively charged in basic solution but neutral in acid solution:



To test this idea, we titrated a suspension of 40 mg Allende DM diamonds in 5 ml 0.002 M HCl with 0.1 M NaOH, metered by continuous, timed flow through a capillary (Fig. 2). (The technique was tested on mixtures of $\text{CH}_3\text{COOH-H}_3\text{PO}_4$ and $\text{HCl-KHC}_6\text{H}_4(\text{COO})_2$.) The pH remained low until the HCl had been neutralized and then rose, but less steeply (solid curve, Fig. 2) than for the HCl supernatant alone (dashed curve), implying some buffering by the microdiamonds. The transition from cloudy white gel to clear yellow colloid occurred between pH 5.0 and 5.6. Five successive titrations of the same sample gave an equivalent weight of $1,700 \pm 200$, corresponding to $(7 \pm 1) \times 10^{-3}$ acidic H^+ per C atom. The error reflects mainly sample losses during re-titration and uncertainties in the definition of the buffering range, not lack of precision in the titration curves.

The differential titration curves (Fig. 2, top) consistently showed two peaks, corresponding approximately to the equivalence points of HCl and microdiamonds. The interval over which significant buffering takes place is much wider than for simple monobasic acids (4.6 as opposed to 1.5–2 pH units), suggesting a range of acid strengths. The data can be resolved *pro forma* in terms of a single pK of 6.4 or, more realistically, as a series of closely spaced pK s ranging from <6 to >9 .

We can get some microscopic information from this macroscopic experiment. Carboxyl carbons have only a single bond available for attachment to the diamond lattice, and can there-

fore be situated only at the corner positions of a cube or at equivalent lattice defects. There are 8 corner atoms in a cube, corresponding to $8/1,060 = 7.5 \times 10^{-3}$ of the carbon atoms in a median-sized microdiamond. This is surprisingly close to the H^+/C ratio of 7×10^{-3} found by titration; some differences would be expected from lattice defects and irregularities in crystal shape, which would provide additional corner positions.

The pK range of ≥ 3 implied by the titration data provides further information. Aliphatic dicarboxylic acids typically have pK s differing by only ~ 1.2 , but the difference becomes larger if the carboxyl groups are close enough to each other (that is, separated by no more than two lattice positions) to interact by hydrogen-bonding or are located near other groups that affect their acidity. The mean spacing of $-\text{COOH}$ groups on median microdiamonds is five lattice positions, and the mean spacing between $-\text{COOH}$ and N is somewhat greater still ($\text{N}/\text{C} \approx 3 \times 10^{-3}$; ref. 13). The observed range of acid strengths implies either some clustering of $-\text{COOH}$ and N, or other variations in the atomic environment of the $-\text{COOH}$ groups (such as rearrangement of the surface atoms to a π -bonded structure¹⁴).

The $-\text{COOH}$ groups cover only a small fraction of the surface sites ($\sim 3\%$ for a median crystal), but nonetheless make the diamonds strongly hydrophilic. In aqueous (or alcohol) media, they form a very bulky gel, which contains of $\sim 97\%$ solvent by volume even after centrifuging, and dries to brown, translucent flakes. The low density observed by Lewis *et al.*² ($2.22\text{--}2.33 \text{ g cm}^{-3}$, compared with 3.52 g cm^{-3} for crystalline diamond) presumably implies the presence of residual water, and perhaps also the amorphous, diamond-like phases a-C and a-C:H (refs 15, 16), which have densities of $2.0\text{--}3.5$ and $1.0\text{--}3.0 \text{ g cm}^{-3}$, respectively.

We must ask, of course, whether the $-\text{COOH}$ is an original feature of the microdiamonds or is a laboratory artefact caused by HClO_4 oxidation of surface atoms. Ion-microprobe analysis of a similar sample, Allende CJ, gave ~ 0.3 atom per cent oxygen¹⁷ (only $\sim 1/5$ of the value implied by the H^+/C ratio, but with an error large enough to accommodate the discrepancy) with a very light isotopic composition ($\delta^{18}\text{O} = -16$ to -27% relative to standard mean ocean water (SMOW)). These data suggest an extraterrestrial origin but do not prove it, because the oxygen isotopic composition of HClO_4 is not known. However, Allende DM also contains 10–40 at% hydrogen, with an apparently extraterrestrial signature⁸ ($\delta D_{\text{SMOW}} = 180 \pm 11\%$), and it therefore seems likely that at least part and perhaps all of the oxygen is also extraterrestrial. The corner atoms of an interstellar diamond grain, having three dangling bonds, would tend to react preferentially with any atoms colliding with the grain, yielding a final distribution that reflects the cosmic abundances and binding energies of these atoms. On these ground a substantial fraction of the corner atoms should be converted to carboxyl groups.

Infrared spectrum

A Fourier-transform infrared spectrum of Allende DM1 in KBr (Fig. 3) shows several peaks, most of which are not present in macroscopic terrestrial diamonds. We suggest the following tentative identifications.

The O-H peak at $3,402 \text{ cm}^{-1}$ comes in part from adsorbed water (as indicated by a KBr blank), but mainly from $-\text{COOH}$ and perhaps other groups containing O-H. The doublet at $2,919$ and $2,849 \text{ cm}^{-1}$ corresponds to the asymmetric and symmetric C-H stretch bands; the presence of sp^3 -bonded hydrogen has been inferred from electron energy-loss spectroscopy⁷ as well as ion-probe⁸ analyses.

The prominent C=O peak at $1,774 \text{ cm}^{-1}$ could be due to $-\text{CHO}$, in addition to $-\text{COOH}$, but as the ion-probe analysis shows barely enough O to account for the H^+/C ratio of 7×10^{-3} , it seems likely that $-\text{COOH}$ is the principal if not the sole contributor.

The broad peak at $1,050\text{--}1,250 \text{ cm}^{-1}$ is in the right range for

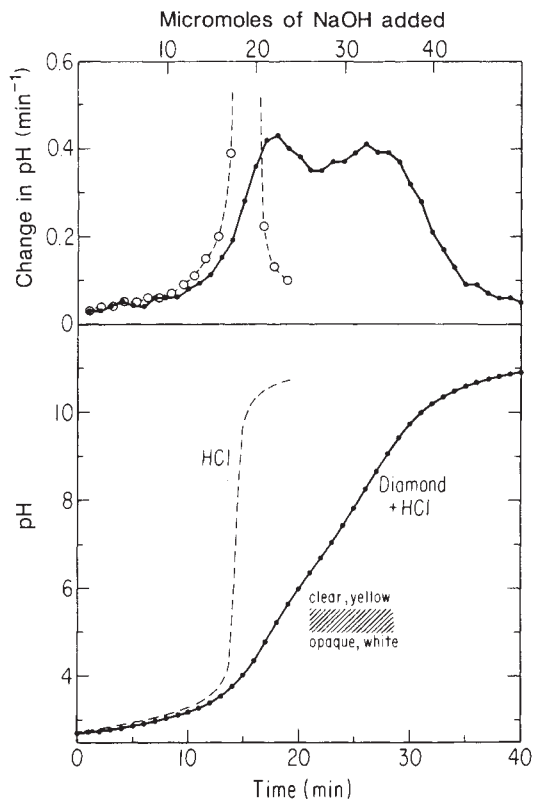


FIG. 2 Titration of microdiamonds in 0.002 M HCl. The curve for Allende DM1 diamond (solid line) is less steep than for HCl alone (dashed line), implying that the diamond is a weak acid. The transition from a white suspension to a yellow colloid occurs over an interval of 0.6 pH units, indicated by shaded section.

N in diamond, but is nearly an order of magnitude more intense than expected from the N/C ratio in Allende diamond. Either the absorbance is somehow enhanced by surface siting or it contains contributions by some other functional group(s); for example, the C-O stretch frequency lies in this region. Contributions by organic compounds are ruled out by the drastic oxidation treatment.

Murchison GL1, reported by Lewis *et al.*², had similar features, which were appreciably stronger in some instances (such as the 3,150- and 1,400-cm⁻¹ peaks). These features may reflect the gentler temperature history of the Murchison chondrite, although contamination or differences in sample preparation cannot be excluded.

Observability of microdiamonds

To assess the observability of interstellar microdiamonds, we have estimated the dielectric function ϵ of meteoritic microdiamonds as follows. In the visible and ultraviolet we took $\text{Im}(\epsilon)$ to be that appropriate for terrestrial Type Ib diamond with N/C=0.003 (refs 18-20), but reduced by a factor of 2.3/3.52 to allow for the reduced density ($\sim 2.3 \text{ g cm}^{-3}$) of meteoritic microdiamonds. Through the Kramer-Kronig relations, this choice of $\text{Im}(\epsilon)$ was then used to compute a first estimate of $\text{Re}(\epsilon)$ from zero frequency up to the ultraviolet. To this estimate of ϵ we then added contributions from four Lorentz oscillators:

$$\Delta\epsilon(\omega) = \sum_{i=1}^4 S_i [1 - (\omega/\omega_{0,i})^2 - i\gamma_i(\omega/\omega_{0,i})]^{-1}$$

with resonance parameters ($S_i, \omega_{0,i}, \gamma_i$) adjusted to approximately reproduce the observed laboratory absorption spectrum (Fig. 3) of Allende DM1 microdiamonds embedded in a KBr matrix. The resulting dielectric function is shown in Fig. 4.

Using this dielectric function, we calculated the contribution that would be made to the interstellar extinction per H atom if a fraction $f_C = 1$ of the solar carbon abundance ($\text{C/H} = 3.6 \times 10^{-4}$; ref. 21) were present in microdiamonds with the log-normal size distribution found for Murchison GL1.

The result (Fig. 5) shows that even though the infrared features in Allende DM1 are stronger than anticipated for diamond, they are nevertheless not strong compared with the extinction produced by interstellar dust: even the strong feature at $0.177 \mu\text{m}^{-1}$ ($1,770 \text{ cm}^{-1}$) would contribute only $\Delta\tau/\tau \approx 0.3 f_C$, where τ is the optical depth. Given the uncertainties in the observed interstellar extinction in this wavelength region, it is possible for f_C to be as large as ~ 0.3 without violating interstellar-extinction constraints. It should be emphasized that this result is insensitive

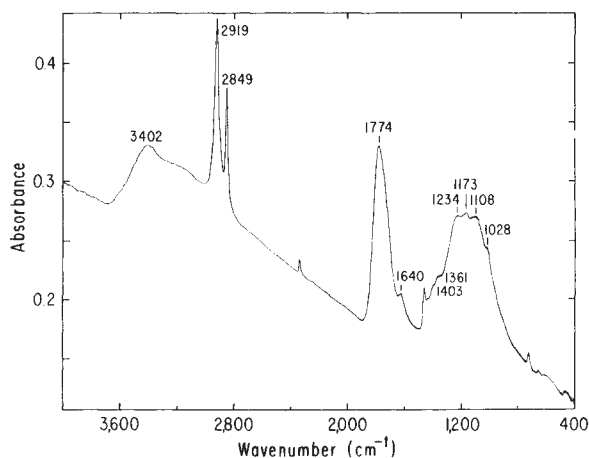


FIG. 3 Infrared spectrum of Allende DM1 microdiamond (density 0.67 mg cm^{-2}). The peaks at $2,919$ and $2,849 \text{ cm}^{-1}$ seem to be due to aliphatic C-H, and the peak at $1,774 \text{ cm}^{-1}$, to a COOH group.

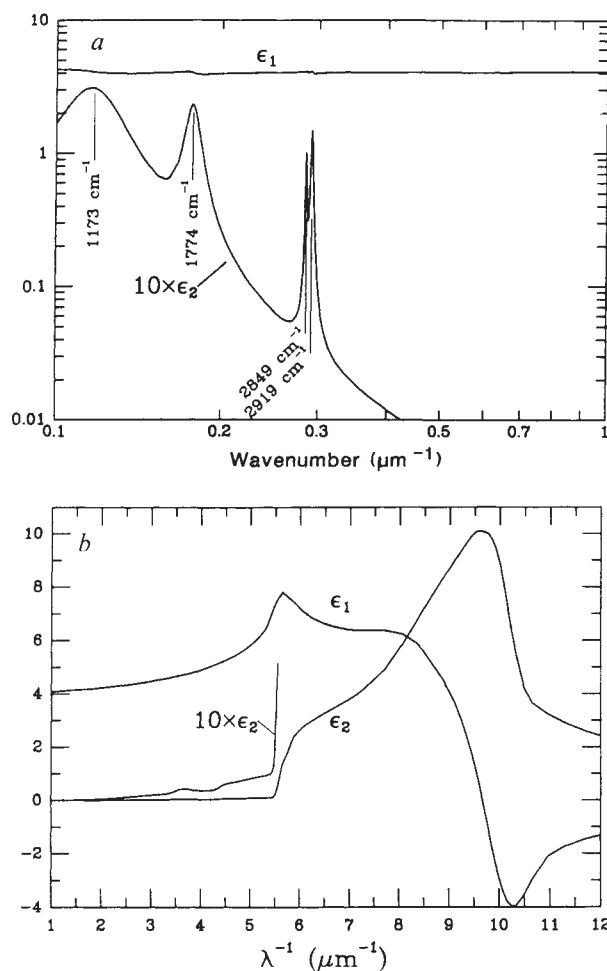


FIG. 4 Dielectric function $\epsilon = \epsilon_1 + i\epsilon_2$ adopted for the Allende DM1 meteoritic diamond in the infrared (a) and visible-ultraviolet (b) (see text).

to the adopted size distribution: provided that $D \leq 1 \mu\text{m}$, the absorption in the $0.177\text{-}\mu\text{m}^{-1}$ region depends only on the total volume of grain material. We have assumed spherical particles; the results are insensitive to moderate departures from sphericity (that is, axial ratios between 0.5 and 2).

The ultraviolet absorption provides stronger constraints. We see that if a fraction $f_C = 1$ was in the form of very small microdiamonds, the resulting extinction at $10 \mu\text{m}^{-1}$ ($1,000 \text{ \AA}$) would exceed the observed interstellar extinction by a factor of about 1.5; at the Lyman limit ($11.0 \mu\text{m}^{-1}$) the excess would be a factor of about 3.5. Accordingly, we can conclude that $f_C \leq 0.1$, so that the microdiamonds would contribute no more than about 15% of the extinction at $10 \mu\text{m}^{-1}$, or about 35% of the extinction at the Lyman limit.

Hecht²² has discussed the strong variation of the microdiamond absorption cross-section arising from the diamond absorption edge at $5.6 \mu\text{m}^{-1}$ (6.9 eV), and has argued that lack of structure in the observed extinction curve implies an upper limit of $f_C < 0.01$; however, we find the upper limit to be an order of magnitude larger. We estimate the differential extinction between 5.6 and $6.0 \mu\text{m}^{-1}$ to be $\Delta\tau/N_H = 2.2 \times 10^{-22} f_C \text{ cm}^2$; with $\tau/N_H = 1.2 \times 10^{-21} \text{ cm}^2$ for the observed interstellar extinction curve, this implies a feature strength $\Delta\tau/\tau = 0.2 f_C$ (a result slightly larger than Hecht's because of our use of a lower density for microdiamonds). To estimate the detection threshold, we note that Carnochan²³ has recently claimed detection of a broad feature at $5.86 \mu\text{m}^{-1}$ with $\Delta\tau/\tau = 0.026$ and a full width at half maximum of $1.2 \mu\text{m}^{-1}$; previous searches²⁴⁻²⁶ for structure in

the ultraviolet extinction curve had failed to disclose any feature at this wavelength. In Fig. 6 we show the effects of adding microdiamond extinction to the observed extinction curve; from these curves it is apparent that even if a fraction $f_C = 0.1$ were in the form of microdiamonds, the onset of microdiamond absorption would be difficult to detect. We conclude, therefore, that as much as 10% of the interstellar carbon could be in microdiamonds without these having been revealed in observational studies of the interstellar extinction. Hecht appears to have overestimated the sensitivity of observational searches for structure in the extinction curve.

Microdiamonds may be detectable in emission. Duley and Williams²⁷ have argued that some of the observed extreme red and near-infrared continuum recently detected from interstellar gas may be luminescence from diamond-like dust, but other candidates for this emission exist, including hydrogenated amorphous carbons²⁸. A secure identification will be difficult without well defined spectroscopic features.

Heating of very small microdiamonds by ultraviolet radiation could result in such emission features. Following absorption of one photon, the particle will be heated to a temperature T_{peak} , which may be high enough for near-infrared emission to occur²⁹⁻³¹ as the grain undergoes radiative cooling. (Indeed, the presence of ~ 90 -atom graphite grains in reflection nebulae, as inferred from such 'quantum heating'²⁹, prompted the re-examination of the ~ 20 -Å carbon grains in meteorites³² that led to the discovery of microdiamonds².) To emit appreciably in a band at wavelength λ the grain must have $kT_{\text{peak}} \geq hc/5\lambda$: $T_{\text{peak}} \geq 350$ K and 510 K for the $1,173\text{-cm}^{-1}$ and $1,774\text{-cm}^{-1}$ bands, respectively. A microdiamond of $D = 16$ Å (250 atoms) absorbing 13 eV of heat will have $T_{\text{peak}} \approx 610$ K, and an appreciable fraction of the radiated energy should appear in the $1,173\text{-cm}^{-1}$ ($8.5\text{-}\mu\text{m}$) and $1,775\text{-cm}^{-1}$ ($5.6\text{-}\mu\text{m}$) bands. An emission feature near $1,160\text{-cm}^{-1}$ ($8.6\text{-}\mu\text{m}$) is observed in a number of objects, but it appears to be narrower than the broad absorption band in Allende DM1 microdiamonds; the interstellar emission feature has been attributed to an in-plane C-H bend in polycyclic aromatic hydrocarbons³³. There is no evidence for an emission feature at $1,774\text{-cm}^{-1}$.

We note that larger microdiamonds do not become hot enough to produce thermal emission in these bands: 13 eV of absorbed radiation will heat a microdiamond of $D > 30$ Å only to $T_{\text{peak}} < 190$ K, which is too cool to emit even at $1,173\text{-cm}^{-1}$. Unfortunately, for the log-normal size distribution of Fig. 1, only $0.03 f_C$ of the carbon is in microdiamonds of $D < 20$ Å; for $f_C \leq 0.1$,

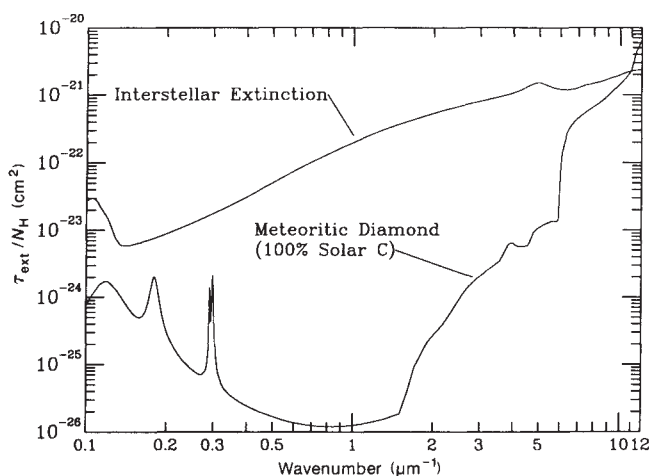


FIG. 5 Contribution to the interstellar extinction that would be produced if 100% of the solar carbon abundance were present in microdiamonds with the log-normal size distribution of Fig. 1, the dielectric function of Fig. 4, and a density 2.3 g cm^{-3} . Also shown, for comparison, is the actual interstellar extinction curve.

microdiamonds small enough to emit in the $1,173\text{-cm}^{-1}$ and $1,774\text{-cm}^{-1}$ bands will probably absorb too small a fraction of the incident starlight for the re-emission to be detectable (although a more detailed calculation of the expected emission would be of value). Thus non-detection of the $1,173\text{-cm}^{-1}$ and $1,774\text{-cm}^{-1}$ bands does not appear to provide a useful upper limit on the abundances of microdiamonds with the size distribution of Fig. 1.

Shock or stellar condensation?

Lewis *et al.*², following Saslaw and Gaustad¹, suggested that the diamonds had condensed as a metastable phase in red-giant atmospheres, because this process, chemical vapour deposition (CVD), had been observed, in the laboratory^{15,34}. Other authors³⁵⁻³⁸ have elaborated on this mechanism.

Tielens *et al.*⁵ have proposed an interesting alternative: collision of graphitic C grains behind supernova shocks. (We use the generic term 'graphitic C' to cover the entire continuum of sp^2 -bonded elemental C, from crystalline graphite to the most disordered amorphous carbon.) As the efficiency of this process is estimated to be only $\sim 5\%$, the diamond should be accompanied by a ~ 20 -fold excess of unconverted graphitic C, with the same isotopic composition and the same noble-gas components. However, no sign of such unconverted graphitic C has been found. The ratio of graphitic C to diamond is < 0.2 for C2 chondrites and ~ 5 for Allende, and this graphitic C not only lacks Xe-HL (an anomalous Xe component enriched twofold in the heaviest and lightest isotopes) and Ne-A2 (a component of $^{20}\text{Ne}/^{22}\text{Ne} = 8.46$ and release temperature $\geq 1,000^\circ\text{C}$) but also fails to match microdiamonds either in $\delta^{13}\text{C}$ (-15% as opposed to -38%) or in $\delta^{15}\text{N}$ (-15 to -20% against -375%). The lack of matching graphitic C cannot be blamed on preferential destruction in the early Solar System, because deuterium-rich, and hence interstellar, organic carbon survived in these same meteorites, despite its greater fragility³⁹. Blake *et al.*⁴⁰ have contended that the 'tightly joined' texture of the microdiamond aggregates is consistent with shock formation, but this texture could easily be a laboratory artefact, produced by drying of the very solvent-rich ($\sim 97\%$) diamond gel. It is possible that interstellar shocks do make some diamonds by the process of Tielens *et al.*⁵, but because meteoritic diamonds do not show its signature, CVD is a more likely alternative.

Conditions in the atmospheres of carbon-rich stars are similar to those required for CVD synthesis of diamonds^{35,38}. Moreover, in the stellar radiation field, transparent grains are heated less than are opaque grains⁴¹, which would permit diamond to form and persist at closer distances than graphite, despite its metastability.

Nuth^{36,37} has raised the interesting possibility that diamond might actually be more stable than graphite at very small grain sizes, if its surface energy is small enough to offset its lower free energy of formation. The actual surface energies, σ , are not well-known, but Nuth calculated that for an assumed σ_g of $4,000\text{ erg cm}^{-2}$, $50\text{-}\text{\AA}$ diamonds could be stabilized if σ_d was $< 5,500\text{ erg cm}^{-2}$, which is well within the literature range of $3,700\text{--}9,800\text{ erg cm}^{-2}$. (Here subscripts g and d refer to graphite and diamond.) This result is somewhat too optimistic, however, as Nuth's calculation did not conserve grains and was done for the unrealistically low temperature of 298 K . The correct relation is

$$\sigma_d < (\rho_d/\rho_g)^{2/3} \sigma_g - D_d \rho_d \Delta G_{f,T}^0 / 6A$$

where ρ = density, D = grain diameter, $\Delta G_{f,T}^0$ = free energy of formation of diamond at temperature T , and A = atomic weight of C. At $T = 1,000\text{ K}$, $\Delta G_{f,T}^0 = 1,423\text{ cal mol}^{-1}$ (ref. 42), so for $D_d = 50\text{ \AA}$ and $\sigma_g = 2,000$ ($4,000$) erg cm^{-2} , σ_d must be less than $1,200$ ($3,900$) erg cm^{-2} . Only the higher value falls within the literature range, and then just barely.

A more serious problem with Nuth's hypothesis is that it predicts formation of microdiamonds wherever elemental

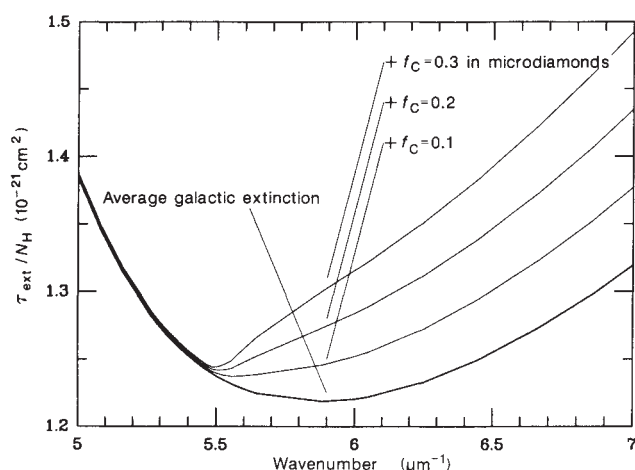


FIG. 6 Extinction in the vicinity of the diamond 'absorption edge'. Shown is the average galactic extinction curve, as well as the extinction obtained by adding a contribution from microdiamonds with 10%, 20% and 30% of the solar carbon abundance ($f_C=0.1, 0.2$ and 0.3).

carbon forms from the gas phase, for example, as soot in fires. Manifestly this does not occur, and although one could argue that graphitic C forms metastably in fires for kinetic reasons, it seems less plausible to invoke metastability for $>10^{13}$ terrestrial fires per year than for a single stellar 'fire'.

Origin of microdiamonds

Clues to the origin of the diamonds come from the diamonds themselves and from their noble-gas components, especially Xe-HL. The diamonds require $C/O > 1$, that is, a carbon star. Xe-HL, on the other hand, requires neutron capture on a fast timescale (in an r-process), that is, a supernova⁴³⁻⁴⁶. The problem is that carbon stars are not massive enough to become (Type II) supernovae; the supernova precursors are more massive ($>8M_\odot$) but carbon-poor stars.

Jørgensen³⁸ has tried to solve this problem by invoking a binary carbon-star system. Matter flows to the more massive star after it has become a white dwarf, permitting it to explode as a Type I supernova. Xe-HL ions in the high-speed ejecta overtake the diamond dust shell produced during the red-giant stage, and implant themselves in the diamonds. Clayton⁴⁶, on the other hand, proposes that Xe-HL is made in a Type II supernova by neutrino-produced neutrons in the helium shell, and that diamonds condense from the expanding shell about a year after the explosion, trapping ambient Xe-HL.

There are three key observations that any model must account for. (1) ^{129}Xe is not enriched relative to the neighbouring

isotopes, although it is made via a chemically active precursor, ^{129}I (half-life $t_{1/2}=16$ Myr). Thus the trapping process must have been chemically non-selective, that is, ion implantation⁴⁷. (2) The $^{136}\text{Xe}_{\text{HL}}/C$ ratio (8.3×10^{-11}) is 2.2×10^{-3} of the solar ratio, implying a very efficient trapping process. (3) Microdiamonds represent about 2% of the total C in primitive meteorites⁹, or $\sim 10^{-3}$ of their cosmic complement of C, implying efficient production and transfer to the early Solar System.

Both models have trouble accounting for some of these observations. The Jørgensen model accounts for (1) but has difficulties with (2) and (3). Because of the long evolution time of low-mass stars, only a small fraction of the diamonds would still be within close range of the supernova at the time of explosion. Moreover, although Type I supernovae are estimated to account for $\sim 2\%$ of the carbon injection into the Galaxy, they show no conclusive evidence for dust⁴⁸. The Clayton model relies on a chemical process (sorption, low-temperature solubility) to trap Xe, and thus fails to explain both (1) and (2); the observed distribution coefficient for Xe in diamond⁴⁹ would require gas densities about 10^{12} times higher than those expected at the onset of condensation in supernova ejecta⁵⁰, namely $\leq 10^{-11} \text{ g cm}^{-3}$.

Evidently a satisfactory model for the origin of microdiamonds is not yet available, although a few elements of such a model can be specified with some confidence, specifically a supernova for production of Xe-HL, ion implantation for trapping of Xe and other noble gases, and perhaps CVD for formation of diamonds. □

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Resolution of the 1,238-keV γ -ray line from supernova 1987A

B. J. Teegarden*, S. D. Barthelmy*, N. Gehrels*,
J. Tueller*, M. Leventhal† & C. J. MacCallum‡

* NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA
† AT&T/Bell Laboratories, 600 Mountain Avenue, Murray Hill, New Jersey 07974, USA

‡ Sandia National Laboratories, Organization 1200, Albuquerque, New Mexico 87185, USA

WE report observations of supernova 1987A from the maiden flight of the Gamma-Ray Imaging Spectrometer (GRIS). SN1987A was observed for a period of 11.1 hours on 1 May 1988. Line emission at 1,238 keV and continuum emission (to be reported elsewhere) from 60 to 800 keV were detected¹. A gaussian line profile gives an acceptable fit to the 1,238-keV line. The best-fit parameters are: flux = $8.5 (+2.3, -2.2) \times 10^{-4}$ photons $\text{cm}^{-2} \text{s}^{-1}$; peak energy = $1,235.4 (+2.2, -2.4)$ keV; full width at half maximum = $16.3 (+6.1, -5.7)$ keV. As the 1,238-keV line is redshifted by 1.1 keV because of the motion of the Large Magellanic Cloud (LMC), we find no evidence for a supernova-produced red- or blueshift in the 1,238-keV line. This is to be compared with model-predicted blueshifts (in the frame of the LMC) of typically 3–4 keV (refs 2–4). Furthermore, our measured linewidth is a factor of about two greater than the model predictions, although the discrepancy represents only two standard deviations. Our line profiles are characteristic of optically thin regions, whereas the intensity implies a mean optical depth of about 2. Fragmentation or non-spherical geometry of the supernova shell are possible explanations of the data.

Instruments on satellites and high-altitude balloons have been tracking X- and γ -ray emission from SN1987A since August 1987 (refs 5–12 and A.C. Rester *et al.*, preprint). Line emission has been detected at 847 and 1,238 keV, as well as a hard continuum extending from 4 to >800 keV (see ref. 13 for a recent review). This radiation results from the decay of radioactive ^{56}Co (half-life of 77.1 days), a daughter product of explosive nucleosynthesis, whose emission is primarily at 847 and 1,238 keV. Downscattering of this line emission in the expanding envelope of the supernova is believed to produce most (if not all) of the hard X-ray and γ -ray continuum^{14–16}.

We have observed γ -rays from SN1987A using GRIS, a new second-generation high-resolution γ -ray spectrometer which provides a significant improvement in sensitivity over currently available instrumentation^{17,18}. The GRIS experiment uses an array of seven large cooled germanium detectors to measure the γ -ray spectrum in the 20–8,000-keV range. The experiment was launched on a high-altitude balloon from Alice Springs, Australia, on 1 May 1988 (day 433 after the explosion) and remained at float altitude for a period of 24 h. The supernova was observed for a period of 11.1 h at an average float altitude of 5.0 g cm^{-2} .

During the remainder of the flight the Galactic Centre was observed for ~ 11 h. The pointing direction was alternated between source and background every 20 min. Background was measured by rotating the telescope azimuth typically through an angle of 180° while holding the elevation constant. The background pointing fields were examined to ensure that no γ -ray sources were contained in them. For the few cases where this occurred, the azimuth offset was adjusted to obtain a clear field. Instrumental background lines were used to verify that there were no systematic differences between target and background pointings. The effective area at 1,238 keV is 46.3 cm^2 , and the experiment field of view is 18° full width at half maximum (FWHM).

The GRIS data in the vicinity of the 1,238-keV line, summed in 5-keV bins, are shown in Fig. 1. The sum of all the 20-min target segments of the supernova is shown in Fig. 1a, and the sum of all supernova background segments is shown in Fig. 1b. There is clearly a significant feature near 1,238 keV, present in the target data but not in the background data. In addition we show in Fig. 1c the sum of all the data in the 1,238-keV region from the second half of the flight (where the primary target was the Galactic Centre). We have found that, within errors, the shape of the instrumental background spectrum in this region remained constant over the duration of the flight. (This behaviour was confirmed with a larger data set from the second flight of GRIS on 28 October 1988).

An instrumental problem during the flight distorted and broadened the instrumental line profiles (see inset in Fig. 1c). This effect, combined with the presence of a strong background line at 844 keV, has complicated the analysis of the 847-keV line. Results on this line will be reported at a later date. The instrumental profile shown in the inset of Fig. 1c displays a main peak, and a satellite peak lower in amplitude by $\sim 30\%$ and in energy by ~ 10 keV. The background line at 1,118 keV (from ^{69}Ge) is consistent with this profile. This is to be contrasted with the broad 1,238-keV feature. Because the supernova line is broader than the instrumental line this double-peaked structure has been smoothed out. A response function was generated using the instrumental line profiles in the range from 800 to 1,800 keV. Using the least-squares fit technique described in Bevington¹⁹, we convolved a gaussian (plus linear continuum) through the instrument response function and fitted the result to the data. The line amplitude, width and centroid, and the slope and offset of the continuum, were all allowed to vary. The fits were carried out over the range 1,160–1,300 keV. Two background-subtracted data sets were used. In the first, only the background obtained at the same time as the supernova observation was used. In the second, the data from the remainder of the flight was added into the background data set. For the latter case the data sets were normalized to the local continuum above the 1,238-keV line.

The results of the fits are summarized in Table 1. Gaussian input profiles produced acceptable χ^2 values for both data sets. Errors for a particular parameter were determined by setting

TABLE 1 Comparison of 1,238-keV measured line profiles with models

	Flux $\times 10^4$ (photons $\text{cm}^{-2} \text{s}^{-1}$)	Peak energy (keV)	FWHM (keV)	FWHM (km s^{-1})
GRIS	8.5	1,236.1†	23.0	5,600
(background from supernova only)	(+3.7, -2.9)	(+4.4, -3.9)	(+12.5 -8.2)	(+3,000, -2,000)
GRIS	8.5	1,235.4†	16.3	4,000
(total background)	(+2.3, -2.2)	(+2.2, -2.4)	(+6.1, -5.7)	(+1,500, -1,400)
10 HMM (refs 2, 22)	9	1,240.2‡	7.5	1,800
Fu and Arnett* (refs 3, 4)	11	1,241‡	11	2,700

* Calculated at day 400.

† Systematic uncertainty = ± 0.3 keV.

‡ Corrected for redshift of the Large Magellanic Cloud.

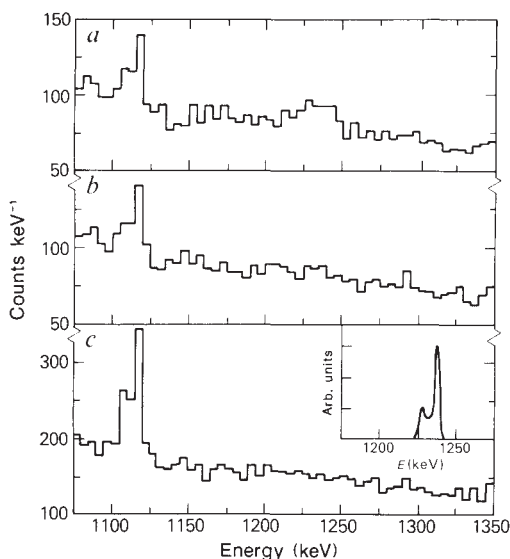


FIG. 1 GRIS spectra in the 1,238-keV region. *a*, Sum of 20-min SN1987A target segments. *b*, Sum of 20-min SN1987A background segments. *c*, Sum of all 20-min Galactic Centre target + background segments. Inset, Instrumental line profile at 1,238 keV derived from a strong calibration line at 1,460 keV.

$\chi^2 = \chi^2_{\min} + 1$ while allowing the other parameters to vary in order to find a minimum^{20,21}. The flux is generally consistent with model predictions. The peak energy is consistent (within errors) with the laboratory value of 1,238.3 keV redshifted by 1.1 keV by the motion of the Large Magellanic Cloud; this is to say, no net red- or blueshift owing to the expansion of the supernova shell is observed. We note that two high-resolution measurements of the 1,238-keV line profile have been reported previously. Mahoney *et al.*¹² reported a peak energy and FWHM of $1,240.8 \pm 1.7$ keV and 8.2 ± 3.4 keV respectively. Rester *et al.* (preprint) reported two peaks at $1,225.5 \pm 1.1$ and $1,239.6 \pm 1.5$ keV with respective widths (FWHM) of 5.6 ± 2.6 and 11.7 ± 3.4 keV. Our results imply a lower peak energy and broader profile than do these earlier measurements. Changing conditions in a dynamically expanding, fragmented supernova shell may account for these variations.

A number of models for SN1987A have been proposed^{2,3,16,22-24}. Bussard *et al.*⁴ have summarized the time-history and line-profile predictions of a representative group of these models. We have chosen two models to test against our data, Pinto and Woosley's 10HMM^{2,22} and Fu and Arnett's Case D (as calculated by Bussard)^{3,4}. Our test criterion is that reasonable fits to the γ -ray line light curve be produced. In Table 2 the measured flux, peak energy and FWHM of the 1,238-keV line are compared against the model predictions. The fluxes are in reasonable agreement with the models. The measured peak energies are, if anything, slightly redshifted, whereas the model peaks are blueshifted. The data suggest that the measured line-widths are broader than the model predictions by roughly a factor of two. (This is significant at the 1–2 σ level depending on the data set and model.) New data obtained from the second GRIS flight in October 1988 (to be reported elsewhere) confirm this general behaviour.

The models we have tested are based on the assumption of spherical symmetry and uniformity of the supernova cloud (as are, in fact, most that have appeared in the literature). To fit the γ -ray light curve it has been necessary to assume mixing of the radioactive material with the more rapidly moving material farther out in the mantle and envelope²⁵⁻²⁷. From the optical light curve, one finds that $0.075 M_{\odot}$ of radioactive ^{56}Ni were produced by the initial explosion¹⁶. Comparison of the radioactive output of the daughter product, ^{56}Co , at day 433 with our data shows that only $\sim 13\%$ of the 1,238-keV γ -rays is escaping

(again, assuming spherical symmetry). If the shell material were uniformly distributed and spherically symmetric, then most of the γ -rays that we observe would be coming from the front side of the shell, which explains the blueshifts displayed by the models. Our data suggest that one (or both) of these assumptions may be incorrect.

If the shell has fragmented, there may be optical paths to the backside material that is moving away from us. Fragmented models can also reproduce the measured γ -ray light curve²⁶. Many supernova remnants display filaments, but it is not known how early they form. If the shell geometry is allowed to depart from spherical symmetry then it may also be possible to reproduce our line profile. Inclined disks or toroids can produce symmetric broadened profiles. Evidence exists for ring-like or toroidal structures in supernova remnants^{28,29}. It remains to be seen whether or not such models can reproduce the γ -ray light curve or the other measured properties of the supernova. $\square$

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Infrared and optical evidence for a dust cloud behind supernova 1987A

James E. Felten* & Eli Dwek

Code 685, Laboratory for Astronomy and Solar Physics, NASA/Goddard Space Flight Center, Greenbelt, Maryland 20771, USA

* Universities Space Research Association

THE 8–13- μm flux from supernova 1987A has increased substantially¹ since around May 1988 (~450 days after the explosion), and the 8–13- μm source has been resolvable¹, having a full width at half maximum of $\sim 1.5''$ in September 1988. This is attributed¹ to re-radiation by an asymmetric circumstellar dust cloud, located

mainly behind the supernova at a distance of ~ 200 light days. This cloud may be related to the gas shell inferred from ultraviolet lines^{2,3}. Here we extend the calculations of Roche *et al.*¹ and consider the further implications of such a cloud. We conclude the following: the expected optical echo from the cloud (*V*-band magnitude $m_v \lesssim 12$) should be resolvable, and this, rather than an internal energy source (such as a pulsar), may cause the observed⁴⁻⁶ slowdown in dimming of the supernova; the reflected light may contribute substantially to the current optical luminosity; observations should be made as soon as possible, for the phenomenon may be evanescent; and the dust spectrum suggests that the dust is carbonaceous rather than siliceous, although the circumstellar region is thought² to be carbon-poor. This cloud behind the supernova may be truly interstellar.

The resolved infrared source around SN1987A seen¹ on day 580 (25 September 1988) after core collapse has been proposed as representing the delayed arrival (echo) of thermal radiation from dust, located a few hundred light days behind the supernova and heated to a temperature $T_d \approx 400$ K by the luminosity of the supernova near visual maximum. A simple calculation shows that the scattered radiation from this dust cloud should also be detectable and resolvable in the optical bands. Let dF_s be the flux of light in the *V* band scattered to the observer *O* at great distance *D* by dust in any small element of volume at *E* (Fig. 1*a*). Normalized to the flux F_{SN} in the same band received at *O* from the supernova at or near its maximum, dF_s observed at time *t* is given by⁷

$$df \equiv \frac{dF_s}{F_{SN}} = \frac{dL_{IR}}{L_{SN}} P(\theta) \frac{Q_a(V)}{\bar{Q}_a(T)} \frac{\omega}{(1-\omega)} \quad (1)$$

where dL_{IR} is the infrared luminosity of the element observed at time *t*, L_{SN} is the intrinsic bolometric luminosity of the supernova near maximum, $P(\theta)$ is the phase function⁸ for visual scattering by the dust at scattering angle θ , ω is the visual albedo, $Q_a(V)$ is the visual absorption efficiency, and $\bar{Q}_a(T)$ is the absorption efficiency averaged over a black-body spectrum at $T \approx 5,500$ K, the effective temperature of the supernova near peak. For a rough approximation we set $Q_a(V)/\bar{Q}_a(T) \approx 1$ (refs 9 and 10). In equation (1) we have assumed that the infrared and supernova emission is isotropic, that the optical depths are small, so that shadowing and multiple scattering are negligible, and that the optical extinctions on paths *SO* and *SEO* (Fig. 1*a*) are equal. The choice of the exact time (near maximum) for evaluating F_{SN} and L_{SN} is not important provided that F_{SN}/L_{SN} depends only weakly on time.

If the emitting dust is concentrated in some finite volume, so that $P(\theta)$ can be replaced by an effective phase function for some average backscattering angle θ_b , then $f \equiv F_s/F_{SN}$ can be approximated by

$$f \approx \left(\frac{L_{IR}}{L_{SN}} \right) \frac{P(\theta_b)\omega}{(1-\omega)} \quad (2)$$

where L_{IR} is the total infrared luminosity from the emitting volume. Typical interstellar dust has¹¹ $\omega \approx 0.7$ and an asymmetry parameter $g = \langle \cos \theta \rangle \approx 0.7$ in the *V* band. If we adopt a Henyey-Greenstein form⁸ for the phase function ($P(\theta) = (1-g^2) \times (1+g^2-2g \cos \theta)^{-3/2}$) and take the backscattering angle to be 150° , then $P(\theta_b) = 0.115$, and $P(\theta_b)\omega/(1-\omega) \approx 0.268$. The observed L_{IR} on day 580 was¹ $\sim 2 \times 10^5 L_\odot$, and L_{SN} at maximum was²⁹ $L_{peak} = 2.22 \times 10^8 L_\odot$, both calculated for a distance of 50 kpc. Equation (2) then gives $f \approx 2.41 \times 10^{-4}$. The *V* magnitude of the scattering cloud on day 580 is then given by $m_s \approx m_{SN} - 2.5 \log f$, where $m_{SN} \approx 2.96$ was the peak *V* magnitude¹² of the supernova (on day 86). The resulting value of m_s is ~ 12.0 . The total *V* magnitude of the supernova on day 580 was 9.52 (ref. 29). Thus about 10% of the light on day 580 should have been due to the scattering cloud. A light echo this bright, spread over a diameter of $\sim 1.5''$, should be resolvable. Apart from uncertainties concerning the dust properties, this estimate, based on the infrared observations on day 580, is quite robust and depends

only weakly on the spatial distribution of the dust. The reflected echo could be brighter. If $\omega \approx 0.5$ and the dust scatters isotropically ($P=1$), then the backscattering is stronger, giving $P(\theta_b)\omega/(1-\omega) = 1$, and the figure of 10% above becomes 38%.

A photometric or spectroscopic search for this optical echo, and an examination of recent data which might reveal its presence, would be worthwhile. Photometric observations are in progress (A. P. S. Crotts and W. E. Kunkel, personal communication). The reported inflection⁴⁻⁶ in the SN1987A light curve, corresponding to a slowdown in the supernova dimming rate beginning around day 600, may be a manifestation of this echo. The reported size of the effect agrees roughly with the estimated 10% above, though it is not yet clear whether the reddish colour reported can correspond to the scattering properties of normal dust.

Ultraviolet lines of N, C, He and O, observed by the IUE satellite, are attributed^{2,3} to a gas shell, possibly spherically symmetric and resulting from stellar-wind interactions, around the supernova. The implied radius, ~ 200 – 300 light days, agrees roughly with the distance inferred¹ for the echoing dust cloud. The latter, however, cannot be spherically symmetric; the time history of the infrared flux does not permit it^{1,13,14}. The dust cloud must be mainly on the back side of the supernova, although it might be a portion of a circumstellar shell. Further observations of the IUE lines and the infrared-optical echo will clarify their relationship.

The history and evolution, from day 580, of the echo and its surface brightness are harder to predict, being model-dependent. The echo may grow in relative (and even absolute) brightness, or it may decline slightly. To illustrate this, we will obtain an exact solution for one simple model. First we note that the infrared data suggest strongly that the dust should be carbonaceous rather than silicate. We have calculated equilibrium dust temperatures and spectra for spherical graphite, silicate and amorphous carbon grains using tabulated optical properties^{10,15-17}. In these calculations we characterize the supernova by a luminosity of $L_{SN} = \frac{3}{4} L_{peak}$, to avoid overestimating the mean dust temperature, and a temperature of $T \approx 5,500$ K. We find that silicate grains with a radius of $0.1 \mu\text{m}$ located at the distance of the dust cloud (200–350 light days) will have temperatures of between 220 and 170 K, and will produce a strong feature at $9.7 \mu\text{m}$. At higher temperatures, reached by very small particles undergoing temperature fluctuations, this feature will be even more pronounced. Graphite and amorphous carbon (at the same range of distances) will attain temperatures of between 470 and 320 K, and will have a smooth spectrum. The relatively

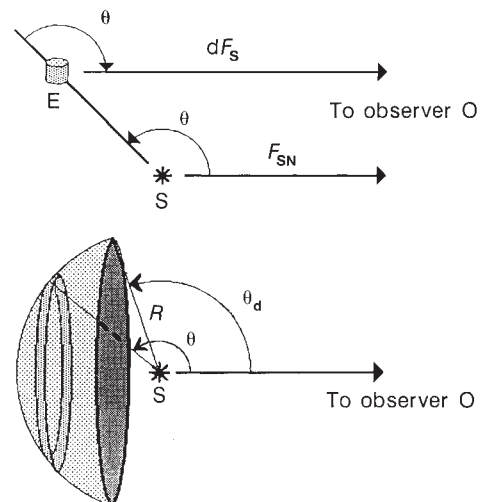


FIG. 1 Geometry of backscatter by *a*, a volume element of dust, and *b*, a spherical cap of dust behind the supernova.

featureless spectrum¹ in the 8–13- μm region suggests, therefore, a paucity of silicates. The absence of silicate does not prove the presence of graphite or carbon dust, but then some other dust candidate must be found. IUE observations² suggest that the circumstellar dust shell is oxygen-rich (that is, the ratio C/O < 1) with CNO abundance ratios of N/C = 7.8 and N/O = 1.6. Dust particles expected to form in such a medium are silicates rather than carbonaceous, contrary to the dust composition inferred from the infrared observations. If, however, the cloud is truly interstellar rather than circumstellar, this apparent contradiction is removed.

Provisionally we assume that the dust cloud is graphitic, and for simplicity we adopt a single grain size of 0.1 μm ($\omega \approx 0.533$, $g \approx 0.302$). We assume that the dust is a thin uniform layer, lying on a sphere of radius R centred on the supernova and taking the form of a symmetric spherical cap behind it (Fig. 1b), thus occupying the range of scattering angles $\theta_d < \theta < \pi$. There is no *a priori* reason why a partial shell should be symmetric about the observer's direction, but in fact the infrared source is observed¹ to be centred on the supernova position to within 0.25". By assuming a shell, we do not mean to prejudice the question of whether this dust is circumstellar or interstellar. We choose a radius $R = 305$ light days, so that the graphite dust, illuminated by $\frac{3}{4}L_{\text{peak}}$, will reach a temperature $T_d = 400$ K, giving a good fit to the infrared data.

For brevity we suppress further discussion of modelling and simply describe our model and representative results. We choose $\theta_d = 112^\circ$. The kinematically accessible paraboloid^{13,14,18} corresponding to the initial supernova outburst (day 0) reaches this edge θ_d on day 420, when the infrared emission first started rising. We approximate the visual light curve by a top-hat function, with a flat summit of $F_{\text{SN}} = \frac{2}{3}F_{\text{peak}}$, dropping to zero at $t_1 = 14$ days and $t_2 = 124$ days. The reflected echo seen on the plane of the sky on day t is a ring with inner and outer radii of $\psi_1(t)$ and $\psi_2(t)$. Using a Henyey–Greenstein $P(\theta)$ function for the graphite, the visual surface brightness at ψ_2 , $\Sigma_v(\psi_2, t)$, and the integrated visual magnitude of the echo, $m_s(t)$, are elementary functions. For normalization we use the observed¹ flux density $S(10\ \mu\text{m})$ on day 580. With a mass absorption coefficient $\kappa(10\ \mu\text{m}) \approx 343\ \text{cm}^2\ \text{g}^{-1}$, the mass of dust involved in the echo is $M_d \approx 3.11 \times 10^{-4} M_\odot$. This echo presents a solid angle $\Omega_{\text{SN}} \approx 2.27$ sr to the supernova, so the graphite mass per steradian of shell is $M_d/\Omega_{\text{SN}} \approx 1.37 \times 10^{-4} M_\odot\ \text{sr}^{-1}$. The optical thickness of the shell for visual scattering is $\tau_s \approx 0.0254$. The infrared luminosity for the model on day 580 is $L_{\text{IR}} \approx 4.5 \times 10^5 L_\odot$.

If gas is also present on this spherical cap, at a gas-to-dust mass ratio of 1,000, then the shell has a total surface density of $\sim 0.137 M_\odot\ \text{sr}^{-1}$. (A ratio of ~ 400 is typical of interstellar matter in the Large Magellanic Cloud¹⁴, but for this circumstellar dust, deficient in silicates, we believe that 1,000 is a better guess.) For comparison, the IUE lines imply² that the near-side gas shell has $\sim 0.1 M_\odot$ of gas spread over ~ 5 sr, that is, $0.02 M_\odot\ \text{sr}^{-1}$. Considering the uncertain mass ratios in the analyses, we might regard these two surface densities as being in approximate agreement. There certainly is more dust on the back side of the supernova, and we might expect that there is also more gas on that side. If there is substantially more, we might expect an upturn in the IUE lines some time after day 420; but no significant upturn has been seen (ref. 3 and G. Sonneborn, personal communication). Analysis¹⁹ of X-ray data implies more gas on the back side, but this gas is closer to the supernova ($r \approx 12$ light days).

On day 580, the outer and inner radii of the model echo are $\psi_2 = 0.92''$ and $\psi_1 = 0.55''$. (This is an acceptable fit to the limited data on the size of the infrared source.) The surface brightness Σ_v at the outer rim is $10.00\ \text{mag arcsec}^{-2}$, and the integrated magnitude is $m_s = 9.88$. This is much brighter than the rough estimate ($m_s \approx 12$) given earlier. Graphite grains produce a greater infrared luminosity for a given 10- μm flux density than

does typical interstellar dust, and also have strong optical backscatter. On day 650, the quantities ψ_2 , ψ_1 , Σ_v and m_s become 0.73'', 0.00'', 10.57 and 10.29 respectively, and on day 700 (23 January 1989) the respective values are 0.48'', 0.00'', 10.90 and 11.32. At this stage the echo is fading rapidly, and on day 734 it should vanish, as the trailing paraboloid passes beyond the dust shell, inducing $\psi_2 \rightarrow 0$. The vanishing of the echo is an artifact of the top-hat function used to approximate the light curve. On the real echo, no point can fade more rapidly than the steepest decline on the supernova light curve. If the dust extends far behind the supernova instead of being limited to a shell, the echo may maintain its brightness or even increase. Our calculation illustrates, however, that changes may be expected over times of weeks or months.

This model calculation also shows that the visual echo corresponding to the observed infrared source can be surprisingly bright. In fact this model is so bright that it can probably be ruled out by inspection of the light curve and spectrum. However, it is not difficult to reduce the brightness of the visual echo. Larger graphite grains or irregularly shaped particles are less efficient backscatterers, and will produce a weaker echo. More detailed calculations are in progress (E.D. and J.E.F., manuscript in preparation). If the visual echo is detected, comparison with the infrared echo will place useful constraints on the optical properties of the dust⁹ and the structure of the medium around the supernova.

Observations have been reported^{20–24} recently of various spatially extended emission features around the supernova, which are possibly echoes. Three reports^{24–26} give possibly conflicting upper limits. The observations in refs 20–26 are in general not contemporaneous, are made in various broad and narrow bands, and describe features in various positions ranging from 0.8'' to 2.5'' away from the supernova. There are suggestions^{20–23} of an axis in the system, possibly bipolar, with position angle $\sim 200^\circ$. Further details of these data are needed before we can say whether an echo corresponding to the infrared source has been detected. A quoted upper limit²⁵ does suggest that the dust must have much weaker visual backscatter than any of the models discussed above. A weak visual echo from the red-giant wind is expected (R. A. Chevalier and R. T. Emmering, preprint).

The possible^{22,23} reappearance of the 'mystery spot'²⁷ must be interpreted with caution, even if the feature is real^{24–26}. Although it is very difficult to accept the original mystery spot as being an echo²⁸, the calculations of Σ_v above show that it is easy to model 'son of spot'^{22,23} as a patch of reflection echo. $\square$

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Unusual olivine and pyroxene composition in interplanetary dust and unequilibrated ordinary chondrites

W. Klöck*, K. L. Thomas†, D. S. McKay* & H. Palme‡

* NASA, Johnson Space Center, Houston, Texas 77058, USA

† Lockheed, 2400 Nasa Rd 1, Houston, Texas 77058, USA

‡ Max-Planck-Institut für Chemie, Saarstrasse 23, 6500 Mainz, FRG

CHONDRITIC porous interplanetary dust particles (IDPs) collected in the stratosphere are considered to be micrometeoritic material different from any class of meteorites because of their fine-grained textures and high porosities¹. Several authors have suggested that chondritic porous IDPs might be cometary dust². Here we report the presence, in both a number of IDPs and in meteorite matrices, of olivine and orthopyroxene grains, low in FeO, but containing up to 5 wt% MnO. The majority of olivines and pyroxenes in meteorites contain less than 0.5 wt% MnO. The presence of these low-iron, manganese-enriched (LIME) olivines and pyroxenes in IDPs and meteorites may indicate a link between the origin and history of IDPs and the matrix material of primitive meteorites. The origin of the LIME silicates could be explained by condensation from a gas of solar composition. Forsterite is the first major silicate phase to condense from a solar nebula gas, and Mn, which is not stable as a metal under solar nebula conditions, would condense at ~1,100 K as Mn_2SiO_4 in solid solution with forsterite^{3,4}.

We analysed 25 chondritic porous dust particles by windowless energy-dispersive analysis⁵ for major elements ($6 < Z < 28$). Twenty of these dust particles were then embedded in epoxy and cut into thin sections using an ultramicrotome equipped with a diamond knife⁶. The section thickness is ~100 nm. Small particles (<50 μm) of fine-grained, opaque, Semarkona, Murchison and EET83226 matrix were processed in the same way. Mineral analyses were performed at 100 kV on a JEOL 100CX analytical electron microscope using the Cliff-Lorimer ratio method⁷. In this method, results can be presented as ratios to any arbitrary major element or as wt% of a total normalized to 100%. We present our data as wt%. The analytical uncertainties are <5% (relative) for major elements and 10–30% for minor elements (that is, those <1 wt% in abundance) as determined by analyses of mineral standards.

The average composition of the bulk particles is similar ($\pm 40\%$) to chondrites, which are, in turn, compositionally similar to the Sun (except for very volatile elements like H, N, O and C) and presumably to the Solar System as a whole⁸. In detail, however, ratios of elements to Si, such as Mg, Al, S, Ca, Fe, Ni and O, deviate from the CI chondrite ratio⁹, but it is not clear if this is caused by enrichment of Si or depletion of the other elements.

Figure 1a shows a correlation of FeO with MnO for olivines and pyroxenes in various primitive-meteorite classes. MnO contents in all cases are <0.5% and show no increase with increasing iron content. There are two exceptions: Mn-rich olivines and pyroxenes have been found in a coarse-grained chondrule rim in the Allende meteorite¹⁰ and a Mn-rich augite has been found in the matrix of the Tieschitz chondrite¹¹.

FeO and MnO contents in olivines and pyroxenes of extraterrestrial dust particles are plotted in Fig. 1b. Figure 1a and b are drawn to the same scale. Most of the analysed Mn-rich olivines and pyroxenes plot above a line separating the 'meteorite' field from the 'IDP' field.

Representative analyses of Mn-rich olivines and pyroxenes in IDPs are given in Table 1. Absolute MnO contents in olivines and in pyroxenes are in some cases even higher than FeO

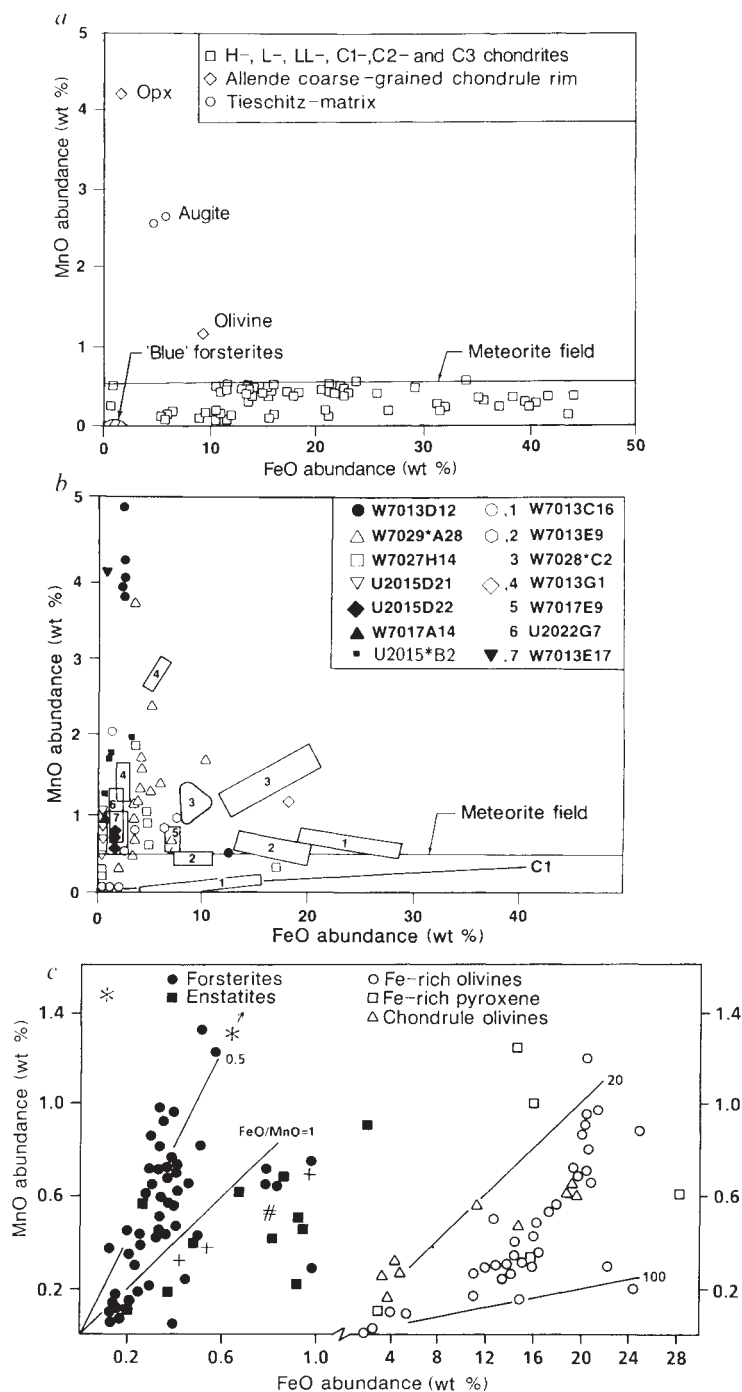


FIG. 1 a, FeO and MnO abundances in olivines and pyroxenes of various undifferentiated meteorites. MnO contents are always below 0.5 wt% and show no increase with increasing iron content. Olivine and orthopyroxene high in Mn are only reported from a coarse-grained chondrule rim in the Allende meteorite and the high-Mn augite was found in the Tieschitz matrix. b, FeO and MnO abundance in olivines and pyroxenes of extraterrestrial dust particles. Most of the IDP olivines and pyroxenes plot at significantly higher Mn contents compared to meteoritic olivines and pyroxenes. (Some olivine and pyroxene data with <2 wt% FeO and <0.5 wt% MnO have been omitted, for clarity of the figure.) c, Composition of olivines in the Semarkona matrix. Two populations of olivines were found in the opaque matrix of the Semarkona meteorite. (1) Fe-rich olivines (fa 2–fa 30, where fa = $Fe/(Fe + Mg) \times 100$), which are similar in composition to chondrule olivines. FeO/MnO ratios of these iron-rich olivines are mainly between 20 and 50. (2) Mg-rich olivines (fa 0.3–fa 0.5) which are characterized by their high Mn content relative to their iron content. FeO/MnO ratios of these forsterites are 0.5–1.0. Olivines similar in composition to these forsterites were found in several extraterrestrial dust particles and in Murchison (CM2) and EET83226 (CM2) matrix material (#, Murchison forsterite; *, EET83226 forsterite; +, EET83226 enstatite).

contents. MnO contents as high as 2.6 wt% in olivines and as high as 5.1 wt% in pyroxenes were observed. The LIME silicates, however, represent only a minor fraction of the anhydrous silicates in IDPs. Most of the particles also contain FeO-rich olivines with FeO contents ranging from 10 to 30 wt% FeO and with MnO between 0.05 wt% and 0.5 wt%. These olivines plot in the meteorite field in Fig. 1a. Obviously, there are also olivines in IDPs, which are compositionally very similar to olivines in chondritic meteorites.

Pyroxenes in IDPs differ from meteoritic pyroxenes not only because of their high Mn contents but also because of significantly higher abundances of Al_2O_3 and Cr_2O_3 compared with unequilibrated ordinary chondrites (UOC) chondrule and matrix pyroxenes (Fig. 2). IDP-orthopyroxenes that are low in MnO are also low in Cr_2O_3 , but are higher in Al_2O_3 content than UOC-chondrule pyroxenes. Some Semarkona matrix pyroxenes plot in the Al_2O_3 - Cr_2O_3 diagram approximately in the same area as IDP pyroxenes. This could indicate that Al_2O_3 and Cr_2O_3 contents in meteoritic low-Ca pyroxenes decrease with increasing degree of equilibration.

IDP pyroxene data differ greatly from achondritic pyroxenes because major and minor elements do not display correlations like those seen in, for example, Pasamonte or Shergotty pyroxenes. In IDP pyroxenes, incompatible elements such as Al and Ti and compatible elements such as Mn and Cr show no systematic increase or decrease with increasing Fe/Fe + Mg

ratio, as is the case for pyroxenes in basaltic meteorites. It is therefore unlikely that pyroxenes in chondritic IDPs are derived from the regolith of a differentiated parent body. High-Mn IDP pyroxenes are also different in composition from the majority of pyroxenes in type 3-6 ordinary chondrites. If Cr_2O_3 is plotted against Fe/Fe + Mg, there is little overlap between IDP pyroxenes and most meteorite pyroxenes.

IDPs are mixtures of fine-grained, non-equilibrium mineral assemblages which can best be compared to matrix material of carbonaceous and unequilibrated ordinary chondrites. Semarkona is one of the least equilibrated ordinary chondrites¹², and it is therefore appropriate to compare olivine and pyroxene compositions of IDPs with those in the matrix of Semarkona. Some representative analyses of Mn-rich forsterites found in the matrices of Semarkona, Murchison and EET83226 are given in Table 2. A graphical presentation of the Semarkona matrix olivine composition, given in Fig. 1c, shows two olivine populations in the Semarkona matrix (see Fig. 1 legend).

The composition of these LIME forsterites is comparable to LIME forsterites in several IDPs (W7029*A28, U2015D21, W7017A14, U2034C10, U2015*B2, W7029B12). The distinct chemical signature of the LIME silicates seems to support a related origin of these Mn-rich silicates in the matrices of Semarkona, Murchison, EET83226 as well as in IDPs.

What is the origin of these unusual olivine and pyroxene compositions? There are two possibilities: reduction of initially

TABLE 1 Representative analyses of LIME olivines and pyroxenes in extraterrestrial dust particles

	W7029*A28		U2015D21		W7027H14	
	Olivine	Pyroxene	Olivine	Pyroxene	Olivine	Pyroxene
SiO ₂	42.95	56.76	42.87	61.24	41.25	59.20
Al ₂ O ₃	ND	1.69	ND	0.19	ND	0.27
MgO	55.84	32.90	55.61	36.46	53.11	39.56
CaO	0.08	1.27	0.05	0.05	0.05	0.08
TiO ₂	ND	0.08	ND	0.07	ND	0.05
Cr ₂ O ₃	0.14	1.55	0.36	0.78	0.17	0.49
MnO	0.67	1.72	0.95	0.87	1.88	0.26
FeO	0.32	4.03	0.16	0.34	3.54	0.09
	U2015*B2		W7013C16		W7029B12	
	Olivine	Pyroxene	Pyroxene	Olivine	Olivine	Pyroxene
SiO ₂	41.89	54.85	54.68	43.10	41.50	55.81
Al ₂ O ₃	0.22	4.01	5.40	0.15	ND	5.06
MgO	57.22	37.00	33.83	56.14	56.18	33.63
CaO	0.11	0.57	1.00	ND	0.06	1.40
TiO ₂	ND	ND	ND	ND	ND	0.17
Cr ₂ O ₃	0.17	0.93	1.60	0.18	0.49	1.81
MnO	0.32	1.78	2.03	0.16	1.01	1.04
FeO	0.07	0.87	1.46	0.27	0.75	1.08
	W7013E17		W7013G1		W7013D12	
	Olivine	Pyroxene	Pyroxene	Olivine	Olivine	Pyroxene
SiO ₂	41.35	57.89	56.07	39.95	41.22	56.15
Al ₂ O ₃	ND	0.77	2.62	ND	ND	2.39
MgO	55.29	36.95	32.60	51.61	54.18	30.16
CaO	ND	1.43	2.02	0.06	0.14	1.90
TiO ₂	ND	0.16	0.18	0.06	ND	0.03
Cr ₂ O ₃	0.18	0.68	1.47	0.76	0.52	1.52
MnO	0.92	0.95	4.14	2.63	1.52	5.12
FeO	2.26	1.17	0.90	4.93	2.42	2.73
	W7017A14		U2034C10			
	Olivine	Olivine				
SiO ₂	41.71	42.65				
Al ₂ O ₃	ND	ND				
MgO	56.60	56.00				
CaO	ND	ND				
TiO ₂	ND	ND				
Cr ₂ O ₃	0.32	0.32				
MnO	0.93	0.82				
FeO	0.44	0.21				

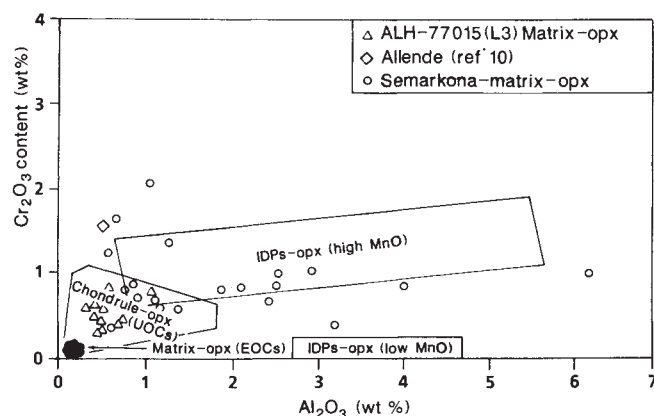


FIG. 2 Comparison of Al_2O_3 and Cr_2O_3 contents in meteoritic pyroxenes and IDP pyroxenes. Pyroxenes of equilibrated ordinary chondrites have very low Al_2O_3 and Cr_2O_3 contents. Slightly higher contents of these elements are found in chondrule and matrix pyroxenes of unequilibrated chondrites whereas strong enrichments of Al_2O_3 and Cr_2O_3 are found in IDP pyroxenes. Some Semarkona pyroxenes plot approximately in the same field as IDP pyroxenes. (EOC, equilibrated ordinary chondrites.)

FeO- and MnO-rich olivines, or condensation of Mn into forsterite and later enstatite in the solar nebula at relatively high temperatures. The first possibility is unlikely, because neither the Mn-rich olivines in the chondritic meteorites nor the IDP-olivines contain any detectable metallic iron inclusions. Also, some of the pyroxenes have such a high Mn content that it is impossible that they ever had an FeO content consistent with this Mn content ($\text{Fe}/\text{Mn} = 30\text{--}100$).

The second possibility, condensation from a solar gas, may be a better explanation. Forsterite is the first major silicate phase to condense from a cooling gas of solar composition. Iron condenses as metal at similarly high temperatures and would react at rather low temperatures (~ 500 K) with forsteritic olivine to produce fayalitic olivine. But Mn, which is not stable as metal in the solar nebula, condenses at $\sim 1,100$ K as Mn_2SiO_4 in solid solution in forsterite^{3,4}. If these MnO-MgO-rich olivines failed to equilibrate with metal at low temperatures, LIME olivines would remain after cooling. The decoupling of Mn and Fe during condensation processes thus provides an effective means for the formation of MnO-rich olivines. Condensation of olivine consumes more Mg than Si, so enstatite will closely follow olivine in the condensation sequence¹³. Enstatite will, of course, also react with the solar gas to incorporate Mn. If conditions are more oxidizing than those predicted for the solar nebula,

some FeO could condense in olivine and pyroxene¹⁴. At the same time, Cr would also condense into the mafic minerals¹⁵. Some FeO and Cr are both observed in olivine and pyroxene of IDPs and meteorite matrices. The Cr/Mn ratios of pyroxenes are lower than the chondritic ratio, implying that some Cr may have condensed as metal. On the other hand, one should not take these estimates too literally, as there is an obvious lack of equilibrium between minerals of a single IDP, in close analogy to what is observed in the matrices of carbonaceous and unequilibrated ordinary chondrites. Furthermore, as no modal estimates are available, it is impossible to define realistically a Mn/Cr or Mn/FeO ratio for a bulk particle.

We therefore do not intend to explain the whole mineral assemblages of single IDPs as condensates. We suggest only that single olivine and pyroxene grains have acquired their high Mn contents during exposure to a gas phase, presumably the solar nebula. Individual grains, especially those with Mn/Mg ratios higher than the solar value, may have formed under different conditions from those with solar Mn/Mg ratios. Very high enrichments in Mn, and other elements, which cannot be explained within the framework of the equilibrium condensation model, require more complex formation processes. Possibilities include fractional or non-equilibrium condensation, and poor kinetic communication between solid and gas.

Our results indicate that the observed LIME silicates in IDPs and in matrices of primitive chondritic meteorites may have a common origin. It seems very likely that they are condensation products from the early solar nebula. They establish a link between chondritic porous IDPs and chondritic meteorites. The source regions of meteorite parent bodies (asteroids) and chondritic porous IDP parent bodies (comets) are believed to be at very different distances from the Sun. The occurrence of mineral phases with identical chemical signature in asteroidal and cometary material could indicate that large-scale dust mixing took place in the early solar nebula. □

TABLE 2 Representative compositions of iron-rich olivines in the Semarkona matrix and of high-Mn olivines and pyroxenes in the Semarkona matrix, in EET83226 chondrule rim material and in the Murchison matrix

	1	2	3	4	5	6
SiO_2	42.33	42.38	41.72	57.48	57.58	58.27
Al_2O_3	ND	ND	ND	1.14	1.95	1.21
MgO	56.41	56.30	56.66	33.01	34.74	33.74
CaO	ND	ND	0.08	6.57	1.71	1.58
TiO_2	ND	ND	ND	0.49	ND	ND
Cr_2O_3	0.22	0.24	0.23	0.49	0.90	1.34
MnO	0.67	0.72	0.98	0.56	1.85	2.66
FeO	0.37	0.36	0.33	0.26	1.27	1.20

	7	8	9	10	11	12
SiO_2	38.77	38.75	41.99	56.96	42.06	56.17
Al_2O_3	0.07	0.20	0.09	1.21	ND	3.78
MgO	42.25	40.19	56.02	37.49	56.24	33.26
CaO	0.11	0.14	0.03	1.39	ND	2.17
TiO_2	ND	ND	ND	0.11	ND	ND
Cr_2O_3	0.49	0.61	0.22	1.00	0.17	1.71
MnO	0.55	0.72	1.51	0.92	0.80	1.71
FeO	17.76	19.39	0.14	0.91	0.73	1.19

1, Forsterite Semarkona ($\sim 1 \mu\text{m}$); 2, Forsterite Semarkona ($\sim 0.2 \mu\text{m}$); 3, forsterite Semarkona ($\sim 0.3 \mu\text{m}$); 4, pyroxene Semarkona ($\sim 0.5 \mu\text{m}$); 5, pyroxene Semarkona ($\sim 0.3 \mu\text{m}$); 6, pyroxene Semarkona ($\sim 0.6 \mu\text{m}$); 7, fayalitic olivine Sem. ($\sim 3 \mu\text{m}$); 8, fayalitic olivine Sem. ($\sim 4 \mu\text{m}$); 9, forsterite EET83226 ($\sim 0.5 \mu\text{m}$); 10, pyroxene EET83226 ($\sim 0.4 \mu\text{m}$); 11, forsterite Murchison ($\sim 0.6 \mu\text{m}$); 12, pyroxene Murchison ($\sim 1.0 \mu\text{m}$).

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Identification of body-centred cubic cobalt and its importance in CO hydrogenation

Saul E. Colley*, Richard G. Copperthwaite*§, Graham J. Hutchings†, S. Petrus Terblanche‡ & Michael M. Thackeray‡

* Catalysis Research Programme, Department of Chemistry, University of the Witwatersrand, PO WITS, Johannesburg 2050, South Africa

† Leverhulme Centre for Innovative Catalysis, Department of IPI Chemistry, University of Liverpool, UK

‡ Division of Materials Science and Technology, CSIR, PO Box 395, Pretoria, South Africa

THE Fischer–Tropsch reaction, involving the hydrogenation of CO to form hydrocarbons and their oxygenated derivatives, is a catalytic process of considerable industrial importance. We have recently¹ studied the process using cobalt/manganese oxide catalysts, and have investigated the nature of the working catalysts under realistic synthesis conditions. Here we report on *in situ* X-ray diffraction studies of the bulk structure of the catalyst during reduction with hydrogen and subsequent hydrogenation of CO. We identify the formation of a body-centred cubic (b.c.c.) phase of metallic cobalt during the process. This phase, unknown until recently and never before prepared in bulk, is metastable; it reverts to the stable face-centred cubic structure after exposure to air and slight pressure at room temperature. The body-centred cubic structure may have a significant influence on the hydrocarbon selectivities of cobalt-containing catalysts.

Our studies of the Fischer–Tropsch reduction have revealed that using cobalt/manganese oxide catalysts with a Co:Mn atomic ratio of 1.0 ± 0.05 produces high yields of propene (C_3H_6) and low yields of methane. This selectivity has been attributed to C_1 and C_2 species on the catalyst surface¹. Electron spectroscopy using an integrated high pressure reactor has identified these carbonaceous species (ref. 2 and R. G. Copperthwaite and M.J. Betts, manuscript in preparation). An appropriate next step is to determine the bulk structure of the catalyst itself.

The XRD data were obtained on a Rigaku Geigerflex D/max IIIA computer-controlled diffractometer equipped with a high-temperature attachment (HTA). Cu ($K\alpha$) radiation (45 kV, 30 mA) was used, with the nickel foil window of the furnace heat shield acting as a ($K\beta$) filter. Powder samples of the cobalt-manganese oxide catalyst (~ 0.7 g by mass) were compacted onto a platinum mesh support, mounted vertically within the HTA chamber. Ultra-pure H_2/CO gas flowed over the catalyst sample at rates of $10\text{--}40$ ml min^{-1} at a pressure slightly above 82 kPa. The temperature of the furnace was controlled with an Ero Electronic MPL programmer to within (nominally) 1°C of the set point.

Samples of mixed cobalt/manganese oxide catalysts were prepared by co-precipitation of the nitrate salt from ammoniacal solution³. Our X-ray diffraction studies indicated that a substantial amount of the mixed spinel M_3O_4 (where M is either Co or Mn, randomly distributed between the octahedral and tetrahedral sites) was present after co-precipitation (Fig. 1a), occasionally together with smaller amounts of a more oxidized phase $CoMnO_3$. These phases persisted after calcination in air at 500°C . Reaction with hydrogen (flow rate of 20 ml min^{-1}) for 16 h at 270°C led to the production of cubic MnO as the only crystalline phase. After a further 6 h at 450°C , a weak peak in the X-ray diffractogram at $2\theta = 44.1^\circ$ was also observed, indicating the onset either of a face-centred cubic (f.c.c.) or a body-centred cubic (b.c.c.) metallic cobalt phase, which could not be unequivocally differentiated at that stage (Fig. 1b).

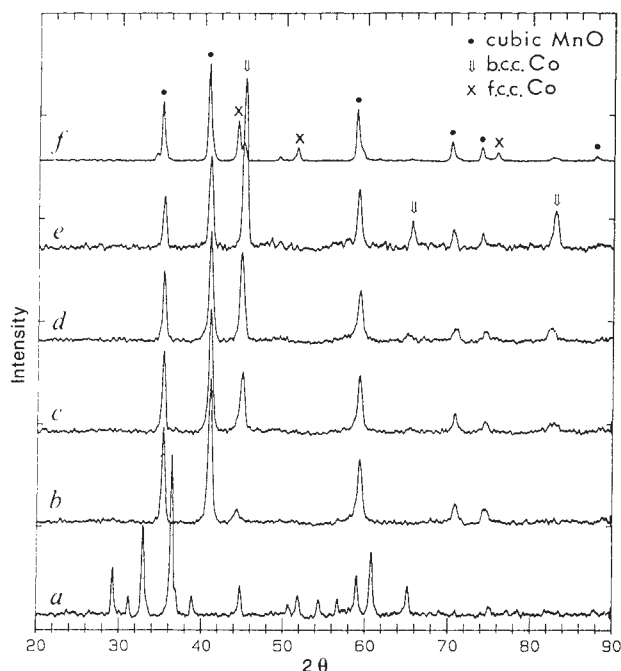


FIG. 1 XRD patterns for the Co/MnO system at various stages of chemical treatment. a, Mixed spinel after calcination; b, after final reaction with hydrogen at 450°C ; c–e, after 24 h, 72 h and 112 h reaction with synthesis gas at 220°C ; f, resulting sample crushed in air.

We suspect that most of the metallic cobalt produced by reduction of the mixed spinel is too finely divided to be observed by powder X-ray diffraction under these conditions and that sintering on the MnO support is not an important factor. The sample temperature was then allowed to stabilize at 220°C and, after 2 h under flowing hydrogen at this temperature (during which no further phase changes were detected), the gas was substituted for a 1:1 by volume ratio mixture of H_2/CO at a flow rate of 40 ml min^{-1} . Previous studies¹ indicate that under such conditions hydrocarbon production from the synthesis gas will begin immediately and that a stable performance is achieved after ~ 100 h. It should be noted that pure MnO has no CO-hydrogenation activity, so that the metallic cobalt observed is thought to be the catalytically active phase. After 6 h of reaction *in situ*, no further phase change was observed, but after 24 h some new peaks of low intensity appeared in the diffractogram (Fig. 1c–e) which grew in intensity; after 112 h these peaks were stronger than those characteristic of the MnO phase. The three new diffraction peaks, at 2θ values of 44.73° , 65.13° and 82.54° , could be indexed to a b.c.c. cobalt phase, with relative intensities similar to those of isostructural α -Fe. The lattice parameter for Co(b.c.c.) was calculated to be 0.286 nm (with MnO used as internal standard after back calibration with silicon). A catalyst sample containing Co(b.c.c.), after reaction for 148 h, was cooled to 20°C under synthesis gas, and the XRD pattern indicated that no structural change had occurred as a result of cooling. Even after exposure of this sample to air for 3 h, XRD indicated the presence of only cubic MnO and Co(b.c.c.). However, by lightly crushing a portion of this specimen in air and leaving it for a further 3 h, XRD revealed that phase transformation to Co(f.c.c.) had occurred (Fig. 1f); this pattern showed, in addition, a few very weak peaks at, for example, 2θ values of $\sim 34^\circ$ and 49° , resulting from an unidentified phase.

Although surface XRD investigations⁴ on sputtered thin films of Co–Cr have provided evidence for Co(b.c.c.) under special conditions of film thickness, our experiments constitute the first reported observations of the bulk formation of Co(b.c.c.). More recent molecular-beam-epitaxy experiments⁵, combined with

§ To whom correspondence should be addressed.

high-energy electron diffraction, have identified Co(b.c.c.) formed epitaxially on GaAs (110) surfaces (in this case a lattice parameter of 0.2827 nm was found). The question arises as to whether the nature of the host matrices, that is, cubic MnO (in our case) or Cr or GaAs, influences the formation of the metastable Co(b.c.c.). To investigate these aspects further we have repeated our *in situ* XRD experiments using the spinel oxide Co_3O_4 , containing no manganese. We observed again the formation of Co(b.c.c.) under the same conditions of temperature and gas composition as for the mixed Co:Mn spinel. In this experiment, the initial reduction product was clearly Co(f.c.c.), with Co(b.c.c.) appearing after ~60 h of reaction with synthesis gas. However, the Co(b.c.c.) phase never completely replaced the Co(f.c.c.) phase. We believe that the presence of interstitial carbon (formed by dissociation of CO) may play an important role in the formation of the metastable Co(b.c.c.) structure; this is supported by some of our recent X-ray photoelectron spectroscopic results (R. G. Copperthwaite & M. J. Betts, manuscript in preparation) on the Co/MnO system.

The formation kinetics of Co(b.c.c.) under hydrocarbon-synthesis conditions correlate well with the activity and selectivities observed as a function of reaction time for our cobalt/manganese catalysts¹. We are now investigating these aspects as a possible route to high selectivities for short-chain alkenes. Some physical properties of the new bulk Co(b.c.c.) phase are also being studied. □

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Flocculation clustering and weakness of ceramics

K. Kendall, N. McN. Alford, W. J. Clegg & J. D. Birchall

ICI, PO Box 11, Runcorn, Cheshire WA7 4QE, UK

It is well-known that the mechanical properties of ceramics are sensitive to flaws which cause weakness^{1,2}. The most damaging flaws are large gas bubbles or particulates which contaminate the ceramic moulding during the compaction process, and which are not healed by sintering^{3–5}. But even when great care is taken to remove such extraneous flaws, for example by filtering or by fabricating under clean room conditions, the strength of the ceramic does not rise to the level expected from the fine structure of the material (M. Real, personal communication), suggesting that some more fundamental intrinsic problem limits the strength. Here we describe a new phenomenon, flocculation clustering, which can account for the low strengths observed. An equation is derived to predict the size of the clusters and is verified by experiments on flocculation of titania sols. We find that flocculation clustering can also be observed in other colloidal systems, such as silica and polystyrene dispersions.

The size of defects in a ceramic can be estimated from the Griffith theory of fracture^{6–8}. The bending strength σ of a ceramic rod has been related to the flaw size f , measured microscopically on a polished surface, by the equation

$$\sigma = 0.737 K_{Ic} / f^{1/2} \quad (1)$$

where K_{Ic} is the fracture toughness of the material. For titanium dioxide ($K_{Ic} \approx 2.0 \text{ MPa m}^{1/2}$) made from an aqueous suspension of pigment particles sedimented for several days to remove all

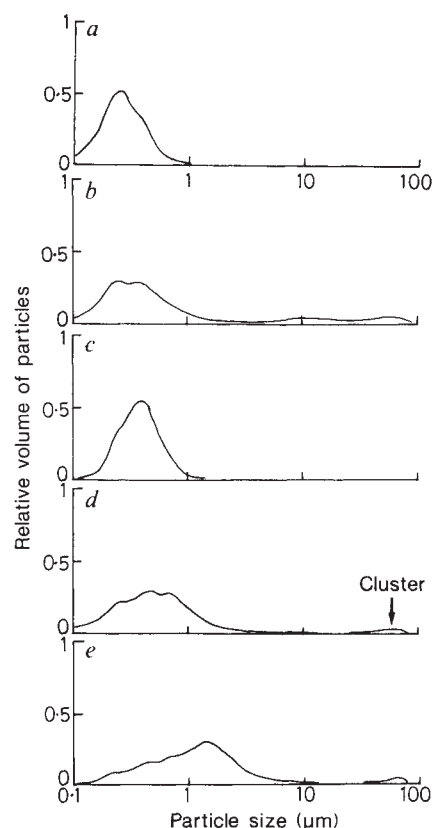


FIG. 1 *a*, Original particle size distribution for stable TiO_2 suspension. *b*, Distribution after drying and remixing in water, showing the irreversible flocculation caused by drying. *c*, After destabilizing with acid, the stable suspension began to flocculate. The curve shows the size distribution after 6 min. *d*, After a further 6 min, the peak corresponding to the primary particles gradually disappeared and a cluster peak became evident at 60 μm . *e*, 80 min later, the small flocs continued to develop but the cluster peak was still evident at 65 μm .

grains larger than 2 μm , one might therefore expect a bending strength of ~1 GPa. In practice, experimental strengths are five times lower than this⁷, corresponding to flaws of ~60 μm in size, unexpectedly large for such a fine-grained powder.

To study the formation of these flaws, the original suspension was tested using the Malvern Mastersizer (MS 1000, Malvern Instruments, Worcester); particle size was measured by laser light scattering, and Mie theory was used to compute the grain size distribution in the range 0.1–600 μm . The original suspension had particle sizes all <2 μm (Fig. 1*a*), as confirmed by optical microscopy. But after drying the suspension at 80 °C and resuspending the dried cake in water, larger entities were observed, with a secondary peak at 15 μm and a third peak near 60 μm (Fig. 1*b*). These appeared to be clusters of the primary grains, adhering too strongly to be broken down by stirring. After ultrasonic agitation in the Malvern machine, some of the larger aggregates disappeared, particularly the 15- μm clusters, but the peak near 60 μm persisted, indicating that these large clusters were structured in such a way as to resist breakdown. Scanning electron microscopy on the filtered suspensions revealed rounded aggregates in the 60- μm size range (Fig. 2), whereas none were found after filtering the original stable dispersion.

The growth of these large clusters was observed by destabilizing the original titanium dioxide suspension by the addition of sulphuric acid and studying the change in the size distribution with time (Fig. 1*c–e*). Initially the stable dispersion had a mean size of 0.24 μm , but after 6 min this peak diminished and a new

peak emerged at $0.4\text{ }\mu\text{m}$ (Fig. 1c). After a further 6 min, the particle size distribution became more broad, with a tail reaching to $15\text{ }\mu\text{m}$. The most interesting feature, however, was the sudden appearance of a separate peak near $60\text{ }\mu\text{m}$ (Fig. 1d). This cluster peak was not broken down by ultrasonic agitation. Further growth of the small flocs was observed as time progressed, but the cluster peak remained stable at around $60\text{ }\mu\text{m}$ (Fig. 1e). Although the precise shape of this peak depended on the colloidal conditions, for example ionic concentration, pH and stirrer speed, the peak was always observed near $60\text{ }\mu\text{m}$.

In another experiment, a dried TiO_2 slurry was found to redisperse in water under light ultrasonic agitation to give no aggregates larger than $2\text{ }\mu\text{m}$ (Fig. 3a). This dried powder was die-pressed at a pressure of 150 MPa to produce a compacted pellet with a packing fraction of 0.55. On redispersing this pellet in water under the same ultrasonic conditions as before, the powder was seen to contain a peak of aggregates near $60\text{ }\mu\text{m}$ in size (Fig. 3b). A centrifuged cake of the fully dispersed powder, when cast into a porous mould and dried slowly, also showed a $60\text{-}\mu\text{m}$ cluster peak after redispersing in water.

Why should such large and dominant particle clusters, all around $60\text{ }\mu\text{m}$ in size, be formed during drying, pressing, chemical destabilizing, centrifuging or redispersing of a fine colloidal suspension containing no particles $>2\text{ }\mu\text{m}$ in diameter? The stability of such large clusters may be explained by considering the competing processes of growth and breakdown of the cluster, which is modelled as a close-packed aggregate of equal spheres.

Growth is dominated by the external surface energy U_s of the cluster, as in nucleation theory⁹⁻¹²:

$$U_s = 5.7\Delta^2\gamma_{\text{SL}} \quad (2)$$

for a spherical cluster of diameter Δ containing $0.74(\Delta/D)^3$ particles of diameter D . Here γ_{SL} is the interfacial tension between the solid particles and the surrounding liquid. Breakdown is determined by the internal bonding energy U_i resulting from van der Waals elastic contacts between the particles¹³⁻¹⁵. Each particle contact has an energy $-1.47\gamma_{\text{SL}}[9\pi\gamma_{\text{SL}}D^2(1-\nu^2)/E]^{2/3}$, where E is the elastic (Young's) modulus and ν is Poisson's ratio, so the total internal energy of the cluster is

$$U_i = -6.53(\Delta/D)^3\gamma_{\text{SL}}[9\pi\gamma_{\text{SL}}D^2(1-\nu^2)/E]^{2/3} \quad (3)$$

For the dominant cluster, $d(U_i + U_s)/d\Delta = 0$, giving

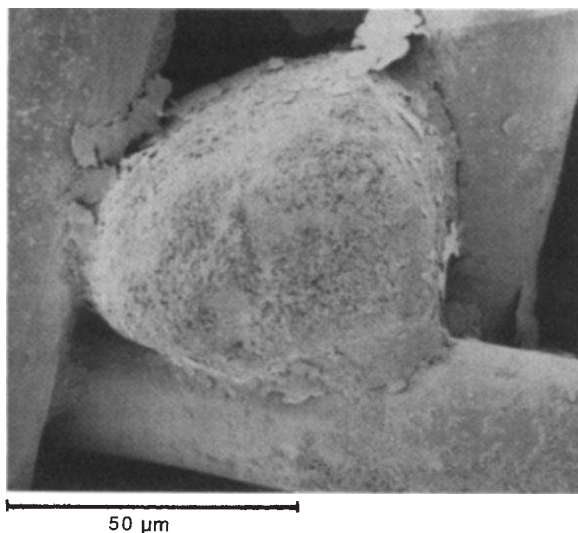


FIG. 2 Agglomerate revealed by filtering a flocculated suspension of TiO_2 using a $50\text{-}\mu\text{m}$ mesh.

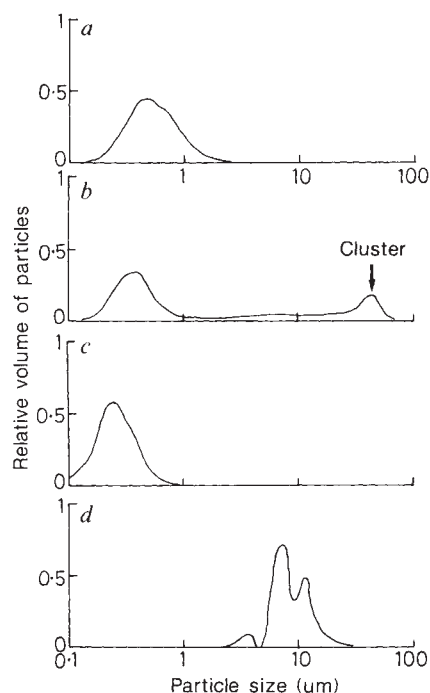


FIG. 3 a, Particle size distribution of TiO_2 powder. b, Size distribution after pressing the powder at 150 MPa and redispersing in water. c, Particle size distribution of polystyrene latex. (Note that the Malvern instrument does not accurately reflect the narrow size distribution for such small particles.) d, Size distribution after flocculation of the polystyrene latex, showing the prominent flocculation cluster.

$$\Delta_0 = 0.064[ED^{2.5}/\gamma_{\text{SL}}(1-\nu^2)]^{2/3} \quad (4)$$

which defines the dominant cluster size Δ_0 .

The predictions of equation (4) were verified by particle-size and flaw-size measurements on sintered ceramic specimens. For titanium dioxide particles (RSM2, Tioxide) of mean diameter $0.24\text{ }\mu\text{m}$, $E = 2.8 \times 10^{11}\text{ Pa}$ (ref. 16), $\gamma_{\text{SL}} = 0.3\text{ J m}^{-2}$ and $\nu = 0.25$, equation (4) predicts a dominant cluster size of $60.3\text{ }\mu\text{m}$. Measurements using the Malvern Mastersizer on a destabilized suspension of this powder gave a peak at $59\text{ }\mu\text{m}$ (Fig. 1d). The suspension was evaporated to produce a dry cake, which was twin-roll-mixed into a 30% aqueous solution of polyvinyl alcohol before being pressed into sheets, dried, cut, burnt-out and sintered at $1,300^\circ\text{C}$ for 1 h, resulting in a ceramic with a bending strength of $235 \pm 34\text{ MPa}$. The toughness of these samples was $2.2\text{ MPa m}^{1/2}$. From equation (1), this result corresponded to a flaw size of $48 \pm 14\text{ }\mu\text{m}$, equal within experimental error to the cluster size predicted by equation (4).

In contrast to these low-strength results, it is known that much stronger titanium dioxide ceramics can be made by applying large shear stresses to the powder suspension to break down the large clusters⁶, or by preparing the powder so that the large clusters do not form¹⁷. In this way, unusually high bending strengths (up to 750 MPa) have been achieved, corresponding to flaw sizes of $5\text{ }\mu\text{m}$.

It is known that titanium dioxide particles precipitated from sulphate solutions are formed by aggregation of primary nuclei into clusters of remarkably uniform diameter¹⁸. Typically, primary nuclei of diameter 7 nm agglomerate into clusters of 200 nm in size. For this system, equation (4) predicts a dominant cluster size of 170 nm , close to the experimentally observed value. It seems possible that equation (4) may explain other instances of unusual nucleation described in the literature^{19,20}.

Stober silica²¹ is another type of particulate suspension that has been shown to form by a clustering mechanism²². The

product particles are not dense, as they would be if grown by accretion from solution, but are porous and of high surface area. Tiny nuclei of silica, ~ 4 nm in diameter, agglomerate to form remarkably uniform spherical clusters of diameter 159 nm. Using the relevant values¹⁴ in equation (4) ($E = 7 \times 10^{10}$ Pa, $\gamma_{SL} = 0.03$ J m⁻², $\nu = 0.3$) predicts a dominant cluster size of 145 nm, close to the experimental value. We have found that large clusters form in silica sols after colloidal destabilization with salt²³.

Growth of clusters was also observed in dispersions of polymer particles. Amorphous monosize spherical particles of polystyrene ($D = 0.244$ μ m, $E = 2.5 \times 10^9$ Pa, $\gamma_{SL} = 0.04$ J m⁻², $\nu = 0.3$) (provided by E. Jones of ICI Colloid Science Group) were suspended in water and gave the particle size distribution shown in Fig. 3c. After destabilizing with aluminium chloride solution, however, flocculation occurred and a large cluster eventually emerged (Fig. 3d) with a mean size of 8 μ m, comparable with the value of 10.3 μ m predicted from equation (4). Previous studies of flocculation in latex dispersions have not reported this clustering²⁴, although in general experimental conditions such as pH may be important. It has long been suspected that emulsion polymerization of styrene and other monomers proceeds by nucleation followed by coagulation to form monosize latex particles²⁵⁻²⁷. Large latex spheres, when studied by electron microscopy, are seen to comprise many smaller spheres stuck together.

Thus, flocculation of colloidal suspensions leads to the self-assembly of rounded, close-packed clusters, predominantly of a specific size, which are orders of magnitude larger than the primary particles. This phenomenon demonstrates how weak colloidal forces acting between fine particles can control the structure and strength of the strongest ceramic solids. Paradoxically, the weaker the van der Waals attraction, that is, the lower the interfacial energy γ_{SL} , the larger are the clusters and the weaker the ceramic as a consequence of these large flaws. Obviously, this argument is only true if the clusters are sufficiently strong to resist breakdown during the ceramic mixing and moulding process²⁸⁻³⁰. The ultimate in weak colloidal forces obtains in biological systems^{31,32} where the surface energy of cells may be as low as 1 mJ m⁻². In this case large cellular clusters, of the order of a few millimetres in diameter, are predicted by equation (4). Such clustering may play a part in biological growth processes. $\square$

Viability costs of male tail ornaments in a swallow

Anders Pape Møller

Department of Zoology, Uppsala University, Box 561,
S-751 22 Uppsala, Sweden

MALE sexual ornaments that increase mating success may evolve even when they decrease other components of fitness such as survival¹⁻⁹. But natural covariation between the apparent level of investment in such ornaments and fitness components such as mating success, fecundity and survival, does not provide incontrovertible evidence that the ornaments are costly, because uncontrolled variables such as overall health⁵ may affect several components of fitness at the same time¹⁰⁻¹². By experimental manipulation of male tail length in the monogamous swallow, *Hirundo rustica*, however, the effects of tail endowment can be tested directly. I show here that in such experiments, females prefer males with elongated tails over those with shortened tails¹³, but that males with experimentally elongated tail ornaments captured smaller, less profitable prey than those with shortened tails. Impaired foraging efficiency of tail-elongated males increased the frequency of fault bars in their tail feathers, probably as a result of food deficiency during moult. Males with experimentally elongated tail ornaments also decreased their natural tail size during moult, thereby causing a fitness loss in terms of delayed breeding and a reduced annual production of offspring resulting from reduced sexual attractiveness during the following year.

The swallow is a monogamous insectivorous passerine of about 20 g in body weight, which forages on the wing and nests in colonies ranging from 1 to 38 pairs in the area studied, at Kraghede, Denmark^{13,14}. Experimental manipulation of male tail length has previously been found to affect the mating success of swallows: females reject males with shortened tails, and experimentally tail-shortened males have longer pre-mating periods¹³. Males with elongated tails acquired mates which laid earlier and produced second clutches more frequently than mates of tail-shortened swallows¹³. Annual reproductive success of males therefore increases with increasing length of their experimentally manipulated tails. Male swallows with elongated tails were also preferred over other males as extra-pair copulation partners¹³. As male swallows with long tail ornaments are preferred by females, further enlargement of this trait must be counteracted by some kind of cost.

To investigate the presence and the extent of such cost experimentally, I manipulated tail length of male swallows during the 1987 breeding season¹³. Male birds were caught at night when they were roosting in their breeding territories, and assigned randomly to one of four groups. The first group (shortened) had a 20 mm piece of feather cut from the middle of the two outermost tail feathers (from 20 mm from the feather base). Apical pieces were glued back using cyanoacrylate 'Superglue' and liquid self-cure acrylic repair material as a catalyst. Mean tail length in this group was reduced by 19.6% to 84 mm. The second group of males (elongated) had the 20 mm pieces inserted into the middle of their outermost tail feathers (20 mm from the base), resulting in an average increase of 16.4% to 125 mm. The third group of male swallows (control group I) had their outermost tail feathers cut in a similar way and the pieces glued back again to control for treatment effects. Mean tail length of this group was reduced by 1% to 103 mm. The fourth group of birds (control group II) were caught and released again without any tail treatment. Mean tail length of this group was 103 mm. The range of manipulated tail lengths (74-138 mm) was very similar to the natural range in the population (84-132 mm; $N = 522$). A total of 44 males were captured shortly after arrival and before acquisition of a mate, whereas 39 males were captured shortly after mating. Swallows from these two groups did

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not differ significantly ($P > 0.20$) in any of the analyses of this paper. I collected additional data on ornament size in swallows in the Kraghede study site during 1984–88.

Swallows feed mainly on dipteran prey during the breeding season, and prefer large prey items over small ones^{15,16}. Large diptera are strong flyers which deftly evade capture attempts by swallows¹⁷. Tail size might therefore be an optimum for efficient prey capture by individuals of a particular body size and condition. Alternatively, swallows might exhibit tails of longer than optimal size, with which they are able to capture prey with reduced but still adequate efficiency. I tested these hypotheses by collecting food brought during calm weather from 09:00 to 12:00 hours to first brood nestlings aged 8–12 days and by recording feeding visits during a one-hour daily observation

period of each pair (up to eight pairs could be watched simultaneously). Nestlings were fitted with neck collars¹⁸ to prevent them from swallowing the food boluses brought to them by their parents. The boluses contained 1–155 insects with 15 insects the average. This sampling procedure did not affect the size of prey brought to nestlings¹⁹. After each visit I removed the bolus from the offspring and recorded the identity of the parent bringing the food. I collected from four to eleven boluses brought by each parent, in total 362 boluses, which were preserved in alcohol. The length of 5,593 prey items was later measured to the nearest 0.1 mm under a light microscope, and body weight was estimated from these body lengths²⁰. Median size of prey brought by parent swallows to their offspring differed among groups of males with different tail-manipulations ($F = 4.43$;

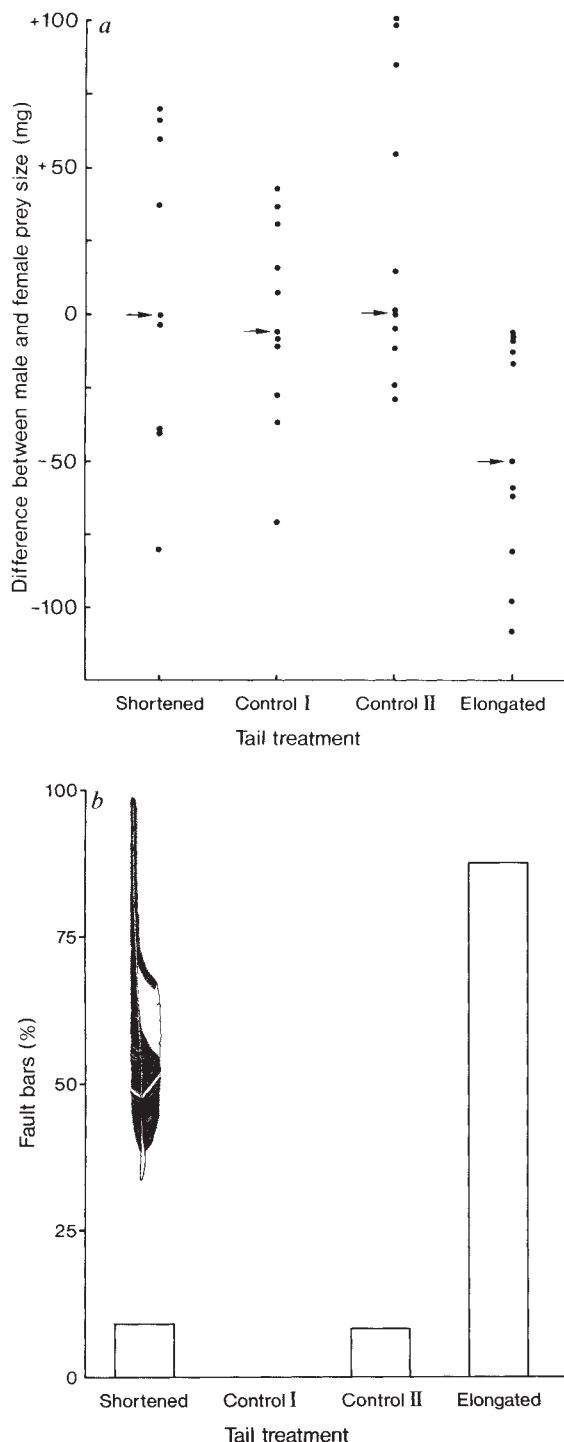


FIG. 1 *a*, Difference in median prey size (mg) taken by males and their mates to offspring aged 8–12 d in their first brood in relation to tail-size manipulation. Arrows indicate medians for each group. The difference in prey size differed among groups ($F = 5.25$, $P < 0.01$). The group with elongated tails differed from all other groups ($P < 0.02$, Duncan's multiple-range test), whereas other groups did not differ ($P > 0.10$). *b*, Proportion of male swallows having fault bars in their tail and wing feathers in relation to tail-size manipulation during the preceding breeding season. The thin white bar (arrow) on the drawing of the feather represents a fault bar. Sample sizes are 11, 9, 12 and 8 for the four groups. Frequency of males having fault bars differed among groups ($P = 3.94 \times 10^{-6}$, Fisher exact probability test). All pairwise comparisons between males with elongated tails and other groups of males were statistically significant ($P < 0.01$, Fisher exact probability test), whereas all other pairwise comparisons were non-significant ($P > 0.10$). *c*, Change in tail length of male swallows from one year to another as a result of moult in relation to tail-size manipulation during the preceding breeding season. Values are means (horizontal lines), $\pm$ s.d. (vertical bars), and ranges (vertical lines). Sample sizes are 11, 9, 12 and 8 for the four groups. Change in tail length differed among groups ($F = 22.29$, $P < 0.001$, one-way analysis of variance). The group with elongated tails differed from all other groups ($P < 0.01$, Duncan's multiple-range test), whereas all other comparisons were non-significant ($P > 0.10$). Tail length did not differ among groups before treatment ($P > 0.10$, analysis of variance), but did so after moult ($F = 4.41$, $P < 0.01$).

TABLE 1 Effect of tail treatment of male swallows during one breeding season on fitness components during their following season

Fitness component	Tail treatment				(P)
	Shortened	Control I	Control II	Elongated	
Survival rate (%)	50.0	42.9	60.0	40.0	(>0.10)
Duration of pre-mating period (days)	2.6 ^a (2.7)	2.6 ^a (2.0)	2.5 ^a (1.8)	8.5 ^b (6.7)	(<0.01)
Proportion having second clutches (%)	63.6	55.6	58.3	25.0	(>0.10)
Fledglings per male	7.3 ^a (1.6)	7.1 ^a (2.8)	7.3 ^a (2.4)	4.0 ^b (1.9)	(<0.01)
Sample size	11 (22)	9 (21)	12 (20)	8 (20)	

Values are percentages (survival, proportion having second clutches) or means (1 s.d.) (other fitness components). *P* values calculated from a G-test (survival, proportion of males having second clutches) or from a one-way analysis of variance (other fitness components). Mean values for treatments are significantly different from each other if they have different superscripts (a and b) ($P < 0.05$, Duncan's multiple-range test). Sample sizes are number of survivors and, in parentheses, number of males originally manipulated.

$P < 0.01$, one-way analysis of variance). Males with elongated tails brought significantly smaller prey items than all other groups of male swallows ($P < 0.05$, Duncan's multiple-range test).

Females did not differ among male tail-manipulation groups in the size of prey offered to their offspring ($F = 1.10$; $P > 0.30$, one-way analysis of variance). As weather, time of day and time of season all affect the kind of prey available to foraging swallows¹⁵, the most appropriate comparison is between males and their mates foraging at the same time. Males with elongated tails brought smaller insects than their mates, whereas sexes did not differ in the size of prey taken for any other treatment group (Fig. 1a). Male swallows with elongated tails were more successful in obtaining extra-pair copulations than others, although males of all four groups attempted such extra-pair copulations at a similar rate¹³. Males with elongated tails may thus have put more effort into trying to get additional mates, rather than providing food for their offspring. However, this is not a plausible alternative explanation because extra-pair copulations exclusively take place during egg-laying and incubation periods of female mates and not during the nestling period²¹.

Male swallows with elongated tails might catch fewer insects than other males if impaired in their foraging ability by the tail manipulation. But males with elongated tails brought more insects per food bolus than other males ($F = 3.60$; $P < 0.05$). They also brought more insects per bolus than their simultaneously foraging mates, although this was not the case for the other groups of males ($F = 6.72$; $P < 0.001$). Median food bolus weight did not differ between experimental groups of males ($F = 1.10$, n.s.), nor did male swallows with elongated tails differ from their mates in median bolus weight more than any other group ($F = 1.26$, n.s.). Mean feeding rate per male swallow during the entire nestling period did not differ between treatments ($F = 0.77$, n.s.), and males with elongated tails did not differ in feeding rate from their mates more than any other experimental group ($F = 0.35$, n.s.). In conclusion, males with elongated tails worked harder than any other group of males.

Swallows moult once a year during a six-month period in their African winter quarters²²⁻²³. If suffering from energy or nutrient deficiency during moult, birds acquire light, so-called fault bars in their feathers, and these sites are especially prone to later breakage²⁴. On average 4.8% ($N = 522$) of the unmanipulated male swallows in my study area were found to possess such fault bars in their tail and wing feathers, short-tailed males more frequently (tail length 86–105 mm: 9.4%; $N = 202$) than males with intermediate and long tails (tail length 106–115 mm: 2.1%; $N = 242$; tail length 116–132 mm: 1.3%; $N = 78$; $G^2 = 13.77$; $P < 0.01$). Tail treatment significantly affected the frequency of fault bars because males with elongated tails were significantly more likely to have bars in 1988, whereas control or tail-shortened males rarely had any (Fig. 1b).

Although the tail length of a given male swallow in consecutive years is to a certain extent repeatable²⁵ ($r = 0.75$ (s.e. = 0.16); $F = 7.05$; d.f. = 48, 49; $P < 0.001$), in unmanipulated males the

tail length does change between years by between -4 and $+18$ mm ($+4.8$ mm on average; 1 s.d. = 4.9; $N = 63$). Long-tailed unmanipulated males increase more in tail length than short-tailed male swallows: the reduced major axis coefficient²⁶ from tail length in year ($i + 1$) regressed on tail length in year (i) was significantly larger than unity (regression coefficient = 1.10 (s.e. = 0.04); $t = 2.66$, d.f. = 61; $P = 0.01$). Experimental manipulation of tail length caused a decrease in pre-manipulation tail size after moult among males with elongated tails, whereas males of all other groups increased the size of their tails (Fig. 1c).

Tail manipulations of male swallows did not significantly affect survival rates of males (Table 1), although survivors with elongated tails had longer unmanipulated tails than non-survivors (survivors: 114.3 mm, 1 s.d. = 11.3, $N = 8$; non-survivors: 102.3 mm, 1 s.d. = 11.0, $N = 12$, $t = 4.80$, $P < 0.001$). Surviving and non-surviving males did not differ in unmanipulated tail length in any of the other three groups ($P > 0.30$). Long-tailed males were thus better able to carry an elongated tail ornament than short-tailed male swallows.

The size of tail ornaments in male swallows strongly affects, through female choice, subsequent mating and reproductive success¹³. Males with elongated tail feathers during the previous reproductive season had more difficulty in attracting a mate as they experienced significantly longer pre-mating periods than all other males (Table 1). All males in the end did acquire a mate, but females who mated with males with previously elongated tails tended to have second clutches less frequently than other females (Table 1). Annual reproductive success measured as the total number of fledglings produced during both first and second breedings differed significantly among groups, and males with previously elongated tails had significantly reduced reproductive success compared with males from other groups (Table 1). Reduced reproductive success of males with previously elongated tails was probably due to acquisition of mates of inferior non-heritable quality^{1,27,28}, expressed as low double-clutching ability.

Tail ornaments in *H. rustica* appear to be costly traits maintained by female choice. Males breeding for the first time seem to invest heavily in tail growth during moult in order to increase their sexual attractiveness²⁹; they are the only group of males with a high frequency of fault bars in their feathers. The evidence presented here indicates that models of sexual selection should take into account the variation in the male's ability to bear the costs of developing and carrying ornaments, because these differences can apparently give rise to a positive association between the extent of development of the ornament and one or more components of fitness^{7,8,30,31}. □

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Disparity curvature and the perception of three-dimensional surfaces

Brian Rogers & Ron Cagenello

Department of Experimental Psychology, University of Oxford,
South Parks Road, Oxford, OX1 3UD, UK

BINOCULAR stereopsis provides information about the relative distance of objects from the differences in the horizontal position of their images on the two retinas^{1–3}. Because the size of the disparity between two points is inversely related to the square of the viewing distance, it is usually assumed that disparities have to be scaled according to distance using the vergence angle of the eyes, or by using the small vertical disparities that also exist between corresponding points of the two images^{4,5}. Here we present evidence that the visual system could extract information about the shapes of surfaces (without the need for scaling) by using the second spatial derivative of disparity—disparity curvature—which remains invariant with viewing distance⁶. Rather than computing the second derivative, we suggest that an approximation to disparity curvature could be derived from the differences in curvature of corresponding line elements in the two eyes.

How good are human observers at detecting and discriminating curved surfaces, and to what extent is this performance

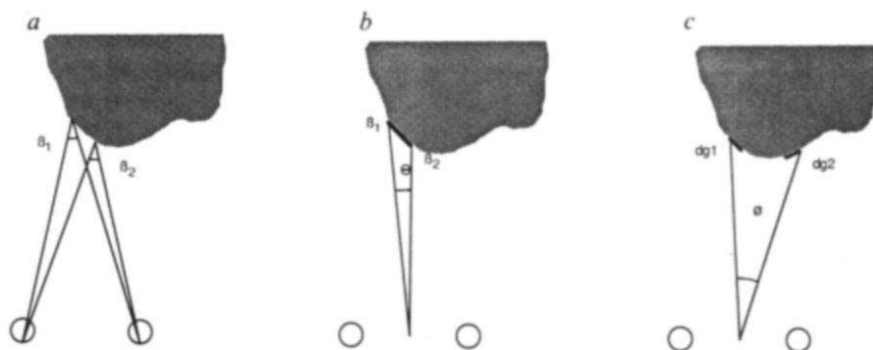
consistent with the use of mechanisms which compute disparity curvature? In our first experiment, observers were asked to judge whether a horizontally orientated cylindrical surface covered with random dots was convex or concave with respect to the observer, using a forced-choice paradigm. Observers were able to discriminate the direction of surface curvature reliably when the surface had a disparity curvature of less than $0.02 \text{ min deg}^{-2}$, corresponding to a radius of curvature of more than 350 cm at the 57 cm viewing distance. Moreover, the disparity range between the closest and furthest parts of the surface at threshold was only one third of that required to discriminate the direction of surface slant over the same spatial extent⁷.

In a second experiment, observers were asked to judge which of two cylindrical surfaces appeared to have the greater perceived curvature. Discrimination thresholds were measured for surfaces with a 256:1 range of disparity curvatures from $0.14 \text{ arc min deg}^{-2}$ (a radius of curvature at the peak of the cylinder of 48.5 cm) to $36 \text{ arc min deg}^{-2}$ (radius of curvature of just 0.19 cm). The results show that observers can do the task with considerable precision. The difference in disparity curvature that could be reliably discriminated was between 4–6% of the disparity curvature of the reference surface, over the central range of disparity curvatures (Fig. 2).

In a third experiment, a matching paradigm was used to obtain an estimate of the variability of matched curvature settings. The random-dot reference and test surfaces were presented alternately for 2 s each and observers were required to alter the curvature of the test surface until it appeared to be the same as that of the reference. The standard deviation of the final matched settings was typically 3–4% of the reference disparity curvature and was approximately constant over a wide range of disparity curvatures.

These experiments show that performance at discriminating or matching singly curved surfaces is very good, but is it based on the judgement of surface curvature *per se*, or could there be some other difference between the surfaces which might be responsible for the impressive performance? The parabolic surfaces used in the latter two experiments were terminated in tangential planes which had the same gradient for each of the stimuli in the forced choice series. Hence, observers could not have been using the gradient of the flanking regions as a cue. Second, we found that performance was not impaired when the disparity range of all the forced choice stimuli was kept constant, ruling out the possibility that the discriminations were based on the disparity range of individual stimuli. Finally, we have data from a fourth experiment which show that observers are able to accurately match the perceived curvature of two parabolic surfaces at different distances (57 and 114 cm) from the observer⁸. At the point of a perfect match, the two surfaces had identical object dimensions, which meant that the disparity range of the two surfaces differed by a factor of four and the disparity gradients of the flanking regions differed by a factor of two.

FIG. 1 *a*, The binocular disparity between two points in space is defined as the difference in the vergence angles needed to fixate each of the two points ($\text{Disparity} = \beta_1 - \beta_2$). *b*, Disparity gradient^{19,20} (dg) is defined as the rate of change of disparity over visual angle (in the limit $(\beta_1 - \beta_2)/\theta$). The disparity difference between a pair of points will be roughly one quarter when the viewing distance is doubled ($\text{Disparity} \propto 1/d^2$), but because the angular separation of the two points will be half, $\theta/2$, the disparity gradient will only halve ($\text{dg} \propto 1/d$). *c*, Disparity curvature is defined as the rate of change of disparity gradient over visual angle (in the limit, $\text{dg}_1 - \text{dg}_2/\phi$). Because the disparity gradients of each of a pair of points on a surface will halve with a doubling of viewing distance, and the angular separation between those two points will also halve, the disparity curvature will remain approximately constant.



Given that it is unlikely that observers could judge when the disparity range of the two surfaces differed by a factor of exactly four, or when the disparity gradients of the flanking regions differed by a factor of exactly two, the results suggest that observers were not using these characteristics of the stimuli to make their judgements.

These observations indicate that observers' judgements were based on the curvature properties of the surfaces, but they do not tell us how the visual system computes the relevant information. One possibility is that the visual system could compute an approximation to the second spatial derivative from the differences in curvature between local line elements in the images reaching the two eyes—curvature disparity (Fig. 3). The argument is directly analogous to one put forward by Koenderink⁹, who suggested that an approximation to the differential invariant of deformation in the optic flow field can be obtained from the orientation changes of local line segments.

Is there any evidence that the human visual system makes use of curvature disparities? First, the idea predicts that there

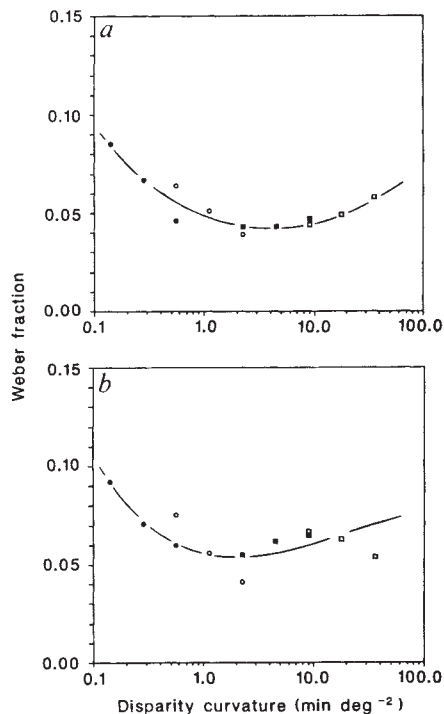


FIG. 2 The Weber fraction, which represents the smallest fractional difference in disparity curvature that can be reliably discriminated (Δ Disparity curvature/Disparity curvature), is plotted here as a function of the disparity curvature of the reference surface, for two subjects: a, BJR; b, RBC. The surfaces used in this experiment were random-dot-covered horizontal cylinders which had a parabolic disparity profile in a vertical direction (see Fig. 3). The upper and lower flanking regions of the parabolic surfaces were terminated with tangential planes, which had the same gradient for each of the surfaces in the forced-choice series to prevent observers using the gradient of the flanking regions to differentiate between the surfaces. The results show that the Weber fraction is remarkably constant over the 256:1 range of surface curvatures used in the experiment, varying from 4–6% for the central range of curvatures, to a maximum of around 9% for surfaces with the shallowest curvatures (on the left). Symbols represent the vertical extent, in degrees, of the entire visible surface, including the tangential flanking areas. $\bullet$, 21°; $\circ$, 10.5°; $\blacksquare$, 5.2°; $\square$, 2.6°. The vertical extent of the curved portion of the surfaces was approximately one-half of the entire vertical extent. The horizontal width of the surface was kept constant at 21°. The ability to see a larger area of the total surface (including the flanking regions) facilitated discrimination of the shallowest surfaces, that is, the difference between the open and closed circles at a disparity curvature of 0.6 min deg^{-2} , but the amount of surface visible had little effect for those surfaces with medium or high curvature.

should be an anisotropy¹⁰ in the thresholds for discriminating the direction of curvature of vertically and horizontally orientated cylindrical surfaces because both the maximum and the average curvature disparities of any local line elements are smaller in the former case. Thresholds were found to differ by a factor of at least 2:1.

Second, this hypothesis predicts that the ability to discriminate or match three-dimensional surfaces should be affected by the characteristics of the surface markings. The maximum curvature difference between corresponding elements on a cylindrical surface curved about a horizontal axis and ruled with lines at $\pm 45^\circ$ (Fig. 3b, d) is less than one half the maximum curvature difference of the same surface ruled with lines at 0 and 90° (Fig. 3a, c). We would therefore predict that thresholds for discriminating the direction of curvature of these surfaces (concave or convex) should be higher in the case of the surface ruled with $\pm 45^\circ$ lines. Empirically, we found the difference to be a factor of 1.3:1 (ref. 11). It is unlikely that this finding is the result of poorer discrimination of curved lines around 45° , an 'oblique effect', because, at threshold, the curvature disparities generated by $\pm 45^\circ$ lines were actually smaller.

Finally, we have found that the discrimination thresholds for vertically orientated cylindrical surfaces ruled with 0– 90° lines are typically higher than those for the same surface ruled with $\pm 45^\circ$ lines. Again, this is consistent in direction, although not in magnitude, with the fact that there are no curvature disparities in the former case. Note that not one of these three predictions would be made by any theory involving the initial extraction of

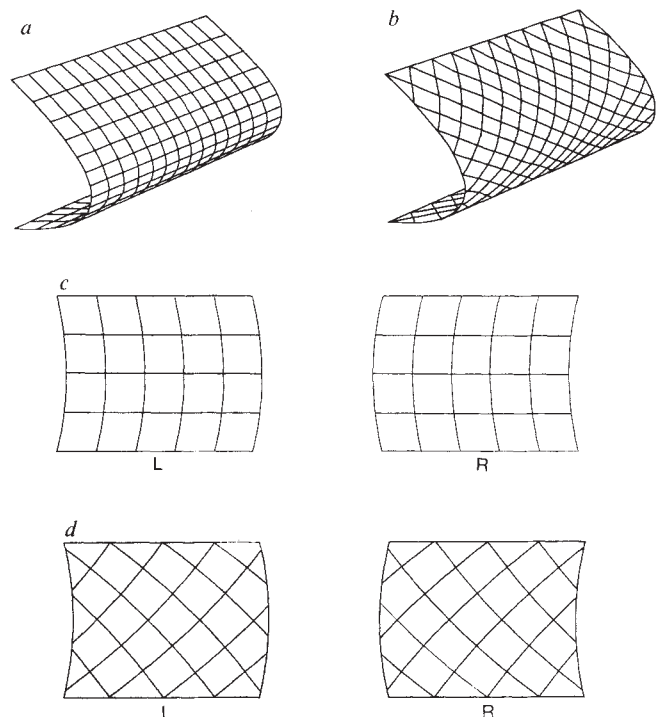


FIG. 3 Two perspective views of a three-dimensional curved surface with a parabolic depth profile (a and b). These surfaces were chosen because the second spatial derivative of the binocular disparities between the two eyes, which we have called disparity curvature, is constant and maximal in a direction orthogonal to the horizontal axis of elongation. Figure 3c, d shows the left and right stereo images of Fig. 3a, b with exaggerated disparities. For a given three-dimensional surface, the largest difference in curvature between corresponding line elements in the two eyes' images, curvature disparity, is generated by a surface with vertical rulings (a). The curvature differences between the stereo images generated by the $\pm 45^\circ$ lines in d are smaller by a factor of >2 compared with those generated by the 0– 90° lines (c).

point disparities, unless it is assumed that there is an anisotropy in the spatial interactions between disparity processes at a subsequent stage¹⁰.

The experimental findings reported here indicate that the ability to match and discriminate three-dimensional curved surfaces is precise and consistent with the idea of using the differences in curvature of local line elements to represent three-dimensional surfaces. The curvature differences involved are very small, but Watt and Andrews have shown that performance in the discrimination of simple curved lines is indeed at a hyperacuity level¹². In terms of the underlying physiological mechanisms, Dobbins *et al.*¹³ and Orban *et al.*¹⁴ have both shown that end-stopped complex cells in monkey visual cortex are selective for the curvature of line stimuli. A representation of the local curvature properties of a surface by itself, which curvature disparity provides, necessarily excludes information about disparity differences and gradients to which we also need access. Consequently, we are not proposing that second derivative descriptions are the only determinants of our perception of three-dimensional surfaces^{15–17}, but our experimental evidence suggests that mechanisms have evolved to exploit the fact that curvature disparity (and its equivalent in the optic flow domain^{6,18}), remain roughly constant with viewing distance and therefore provide direct information about the local curvature of surfaces in the surrounding world. □

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A retina with at least ten spectral types of photoreceptors in a mantis shrimp

Thomas W. Cronin* & N. Justin Marshall†

*Department of Biological Sciences, University of Maryland, Baltimore County, Catonsville, Maryland 21228, USA

†School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK

STOMATOPOD crustaceans, commonly named mantis shrimps, have compound eyes of unique design. A central band composed of six parallel rows of ommatidia separates two peripheral ommatidial groups, and all three regions view the same area of visual space^{1–3}. In the central bands of members of the stomatopod superfamily Gonodactyloidea, four of the ommatidial rows are built of tiers of photoreceptors; in two of these rows, the photoreceptors themselves contain coloured filters⁴. Such a design could in principle produce many spectral classes of photoreceptors using only a single visual pigment^{4,5}. We measured the absorption spectra of the coloured filters and the visual pigments in frozen sections of retinas of a typical species, *Pseudosquilla ciliata*, using end-on microspectrophotometry. The retina contains not one, but as many

as ten visual pigments, each in a distinct photoreceptor class, having maximum absorbances at wavelengths from 400 to 539 nm. Because of the unique anatomy of stomatopod eyes, ten or more spectral types of photoreceptors exist in this species.

The retina of *P. ciliata* has 11 classes of photoreceptors, or rhabdoms, below the level of the distal eighth reticular cell: the single rhabdoms of the peripheral retina and rows 5 and 6 of the central band, plus the two tiers of the main rhabdoms of rows 1 to 4 of the central band (Fig. 1). Rows 2 and 3 of the central band each contain two intrarhabdomal filters. Their absorption spectra resemble those of carotenoid pigments, and have rapidly falling absorbances on the long-wavelength limb (Fig. 2). In both rows, the distal filter (F1) has a 50% transmission point at shorter wavelengths than that of the proximal filter (F2). The filters thus operate in series, permitting light of a greater spectral range to penetrate the distal rhabdom than the proximal rhabdom. Maximum optical densities of these filters were relatively high, ranging from 1.35 to 11.1 density units.

Crustaceans have bistable visual pigments; the photopigment, usually termed rhodopsin, is interconvertible with a thermally stable metarhodopsin⁶. Because only the rhodopsin form takes part in photoreception, however, we needed to know only its absorption spectrum. We therefore maximized rhodopsin content in the retina by dark-adapting experimental animals at least overnight, and commonly for several days (overnight dark adaptation normally produces ~100% rhodopsin in all photoreceptors of crustaceans^{7–9}). The rhodopsin absorption spectrum was then obtained by subtracting the absorption spectrum of the fully photobleached photoreceptor from the spectrum of the same receptor when dark-adapted.

Rhodopsin did, in fact, initially predominate in the retinae. Exposure to bright red light produced a rise in absorption, and a shift of the maximum to shorter wavelengths, in photoreceptors of the peripheral retina and rows 2, 5 and 6 of the central band. Such changes are typical of crustacean rhodopsins having maxima near 500 nm, and reveal the net conversion of rhodopsin to metarhodopsin^{6–10}. Additionally, most photobleach spectra closely resembled template rhodopsin spectra (Fig. 2); it is not possible to fit mixtures of photopigments to rhodopsin templates^{8,10}. The close fits (with two exceptions; see below) also show that the phototreatment produced no stable products having spectra that overlapped the rhodopsin spectrum¹⁰. Photobleaching produced typical density losses of 0.003–0.008 μm^{-1} , similar to average axial densities of rhodopsin in other crustacean rhabdoms^{9,11,12}.

No evidence was found for any pigment photobleaching in the spectral range from 400–700 nm in any rhabdomeres of 8th reticular cells, and it is possible that these contain a visual pigment absorbing maximally at shorter wavelengths. All main rhabdoms, built from reticular cells 1–7, contained photopigments absorbing in this region. The photopigment of rows 5 and 6 of the central band absorbed maximally at 510 nm (Fig. 2). Rhabdoms of the peripheral retina had a second visual pigment absorbing maximally at 498 nm. Moreover, each tier of ommatidial rows 1–4 in the central band contained a different visual pigment. In every case, the rhodopsin of the distal tier had a shorter λ_{max} than that of the proximal tier, usually differing by about 25 nm. This arrangement allows the rhodopsin of the distal tier to act as a long-pass filter. Light entering the proximal rhabdomal region would then be limited to wavelengths absorbed mainly by the long-wavelength limb of the rhodopsin residing there, sharpening the spectral sensitivity.

Overall, the close correspondences of the averaged photobleach data to the rhodopsin template curves indicate that each region possesses only a single photopigment which is actually a true rhodopsin with a retinoid chromophore. The poorest fits occur in row 3, where rhabdoms are particularly difficult to sample because they are densely coated with granules of screening pigment and rarely offer a clear path for spectral scanning

(Fig. 1). The photobleach difference spectrum of the distal tier of row 1 also differs from the template; it drops more rapidly from its peak and has negative values between 450 and 600 nm. This indicates that the photobleaching exposure produced persistent, relatively stable photoproducts absorbing at longer wavelengths than the rhodopsin. A suggestion of the same effect is apparent in the distal tier of row 4.

In determining the dark-adapted spectral sensitivity functions for the sampled retinal regions, it was assumed that absorption by the dioptric apparatus (cornea and crystalline cone) and the short rhabdomere of the 8th retinular cell was negligible, and that each main rhabdom or tier contained no stable photoproducts. When the main rhabdom was untiered, spectral sensitivity was taken to be proportional to the absorbance (fraction of light absorbed) of the visual pigment. This was computed using the best-fit rhodopsin template spectra, the rhabdomal length measured in frozen material sectioned axially, and an estimate of the axial density. As the beam of the microspectrophotometer passed along the axis of the rhabdom, traversing numerous microvillar layers, photopigment axial densities could be estimated directly from the photobleach data. The value used in the computations was that of the largest photobleaches, 0.008 density units per micrometre.

In the tiered rows, determination of spectral sensitivity was complicated by the fact that the light entering each tier was modified by absorption in any overlying photopigments and filters. In rows without filters, the spectral sensitivity of the distal tier was again proportional to the rhodopsin absorbance, and that of the proximal tier was computed by taking the product of the transmittance spectrum of the distal tier and the absorbance spectrum of the proximal tier. Rhabdoms with filters had several successive light-modifying levels, so the sensitivity spectrum of each tier was obtained by iterating this procedure.

The typical, untiered rhabdoms have broad, flat-topped sensi-

tivity functions, ideal for photon capture and for the analysis of polarized light (Fig. 3). These are the tasks for which these retinal regions seem to be anatomically designed⁴. In rows 1–4, however, each rhabdom includes a pair of photoreceptor classes with narrow spectral sensitivities. Except for row 3, these pairs are separated by about 50 nm, which could permit excellent spectral discrimination in the wavelength range between their peaks. Together, the four rows cover the spectrum from 400–630 nm (Fig. 3).

P. ciliata thus possesses a retina with many spectral classes of photoreceptors, produced by a variety of visual pigments which, in combination with stacked photostable filters, modify and spectrally sharpen the light passing through successive retinal tiers. We have examined the retina of another gonodactylid, *Gonodactylus oerstedii*, in less detail, and find that it is organized much like that of *P. ciliata*. One difference, however, is that in *G. oerstedii*, the spectral separations of both the filters and photopigments of row 3 of the central band are greater than in *P. ciliata*. This combination produces a pair of sensitivity functions peaking near 560 and 620 nm.

The diversities of both the visual pigments and the spectral classes in this stomatopod retina are the greatest known in any visual system. Among vertebrates, up to five spectral types of photoreceptors have been found in some fish retinae^{13,14}. The cone photoreceptors of birds and reptiles sometimes contain three or four cone visual pigments together with photostable oil droplet filters, for a total of at least four spectral classes of photoreceptors^{15–17}. Main rhabdoms of crustaceans usually have only a single rhodopsin, absorbing maximally between 450 and 550 nm^{8,9}; another type absorbing at shorter wavelengths may occur in the rhabdomere of the 8th retinular cell¹⁸. Of other invertebrates, flies possess the greatest visual pigment diversity previously known, with at least five distinct visual pigment classes (summarized in ref. 19). Some butterflies have

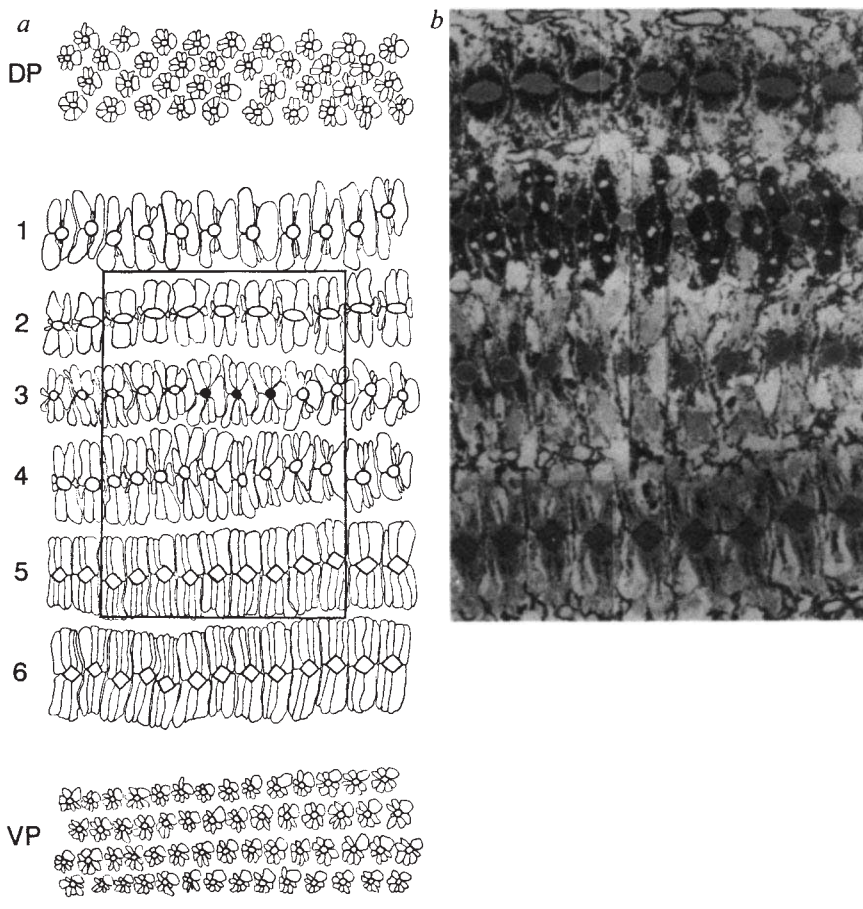


FIG. 1 *a*, Retina of *P. ciliata*. The rhabdoms and retinular cells have been traced from photographs of a fixed and toluidine blue-stained section. The cryosections used for microspectrophotometry were similar but less well organized. The peripheral ommatidia and rows of the central band are indicated. DP, dorsal peripheral retina; VP, ventral peripheral retina; 1–6, rows of the central band. The section was made near the level where the proximal and distal tiers of central band rows 1–4 meet. The dark centres in three ommatidia of row 3 indicate the presence of proximal filters (F2). To the right of the filters, the rhabdoms of row 3 are surrounded by three pigmented cells, which are the retinular cells that build rhabdoms in the distal tier, whereas to the left each rhabdom is surrounded by the four retinular cells that build the proximal tier. Note that the ommatidia of central band rows 1–4 have circular rhabdoms of varying morphology, whereas rows 5 and 6 have similar, square rhabdomal cross-sections. The ommatidia of the dorsal and ventral regions, in the peripheral retina, are all similar and are packed into a typical crustacean hexagonal lattice. Scale bar, 100 μ m. *b*, A section of the photographic montage from which the tracing on the right was made, showing ommatidia of rows 2–5 of the central band. Its location on the tracing is indicated by the rectangle.

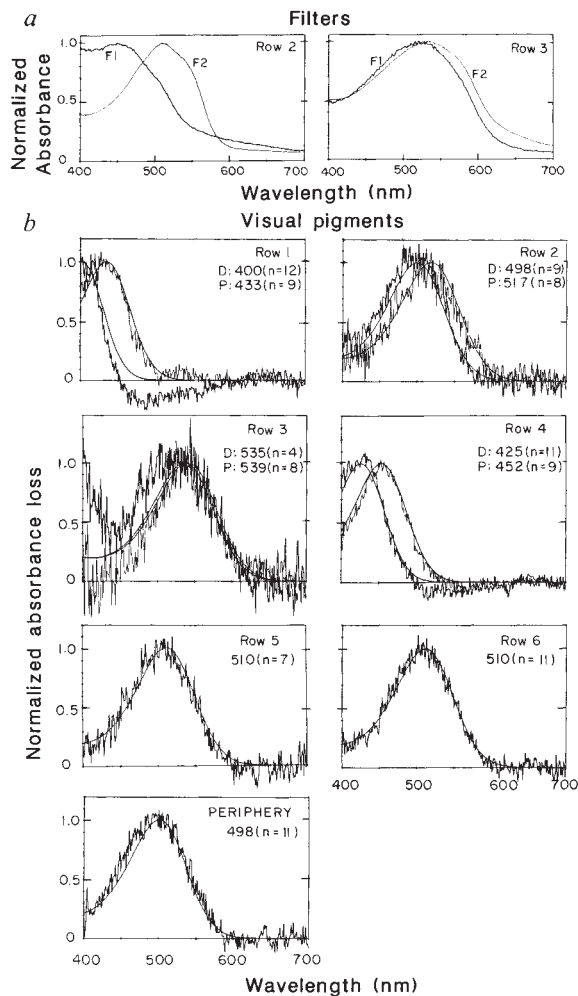


FIG. 2 *a*, Absorption spectra of intrarhabdomal filters of *P. ciliata*. Each spectrum has been normalized to its peak. F1, distal filter; F2, proximal filter. Peak absorbances of the entire filters *in vivo*: row 2 (F1), 1.35; row 2 (F2), 6.1; row 3 (F1), 4.9; row 3 (F2), 11.1. *b*, Normalized, average difference spectra for photobleaching of visual pigments in all retinal regions (jagged traces), and best-fit template curves (smooth traces). The best-fit curves are computed from an 8th-order polynomial derived from vertebrate cone visual pigment data by G. D. Bernard²⁵. Any drifts in the photobleach curves have been removed by setting the average absorbance change from 651–700 nm equal to 0. The number of individual photobleaches included in the average is provided on each panel, as is the $\lambda_{\max}$ of the best-fit rhodopsin template curve (D, distal rhabdomal tier; P, proximal rhabdomal tier).

METHODS. Eyes of fully dark-adapted animals were removed in dim red light and either fixed overnight in 0.5% glutaraldehyde in phosphate buffer (pH 7.4) and subsequently cryoprotected in a solution of 30% sucrose in the same buffer, or quick-frozen with Freon spray immediately after removal. Whole frozen eyes were mounted in a cryostat, and 8- μ m sections were cut under dim red light in the plane transverse to the rhabdomal axes of ommatidia of the central band. Sections were placed in a drop of buffer inside a ring of silicone grease between 2 coverslips and mounted in the microspectrophotometer. The instrument used was of single-beam design; scans were made from 400–700 nm using a circular 1.5- μ m spot of linearly polarized light (see also refs 8 and 9). In cases where the peak optical density of an intrarhabdomal filter was too great for precise determination ($OD_{\max} > 2.5$), scans were taken both of filters occupying the full 8- μ m thickness of the section and of identical filters sectioned only partially. Axial density per μ m was determined by scaling the spectra of the thinner sections to overlap the spectral regions that could be measured from the full sections, and the total density was obtained by taking the product of the density per μ m and the lengths of the filters measured in longitudinal frozen sections. Absorption spectra of the rhodopsins were obtained by placing the 1.5- μ m beam centrally in rhabdoms having unobstructed end-on orientation within the section. The spectrum of the dark-adapted photoreceptor was first measured, the visual pigment was then fully photobleached with a 2–5 min exposure to high-intensity white light. The receptor is considered photobleached when the spectrum could no longer be altered by further exposure to bright white light. In aldehyde fixative, crustacean photopigments photobleach readily^{7–9}; those of *P. ciliata* were equally susceptible to photobleaching in frozen sections from unfixed eyes. A second spectral scan was taken and the difference between the two scans represented the absorption spectrum of the photopigment. Data from photobleaches of 4–12 rhabdoms of each class were averaged, and each visual pigment was identified by subjecting the averaged data to a least-squares analysis of fit to an idealized visual pigment absorption spectrum⁹.

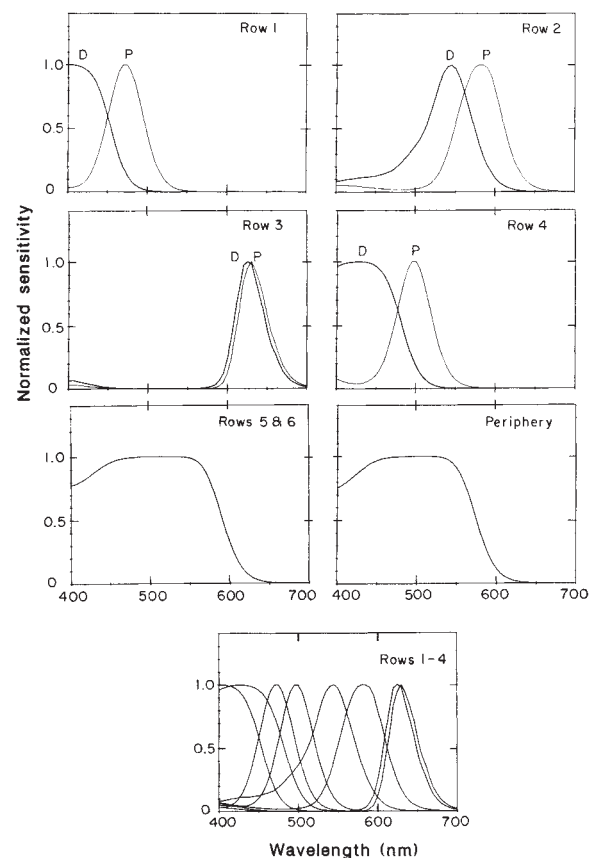


FIG. 3 Normalized sensitivity spectra for all retinal regions, computed as described in the text. Computations use the filter densities of Fig. 2, an axial density of visual pigment of $0.008 \mu\text{m}^{-1}$, and rhabdomal lengths of 150–200 μm for the tiers (rows 1–4), 400 μm for rows 5 and 6 of the central band, and 350 μm for peripheral rhabdoms. The two tiers of ommatidial rows 1–4 of the central band are illustrated in the top four panels (D, distal rhabdomal tier; P, proximal rhabdomal tier), whereas functions for untiered rhabdoms are plotted separately below. The bottom panel includes all sensitivity spectra for the four most dorsal rows of the central band.

pentachromatic vision, produced by an unknown number of visual pigments²⁰.

What advantages are conferred by the presence of such a variety of photoreceptor spectral classes? As mantis shrimps are diurnally active and live in shallow, clear water²¹, they live in a visual environment similar to that of terrestrial animals. Visual pigments characteristically have broad absorption spectra, so that only three or four distinct functional classes can operate in the wavelength band from 400–700 nm^{22,23}. By narrowing the spectral acceptance functions of photoreceptors in rows 1–4 of the central band, *P. ciliata* has beaten this theoretical constraint. Its visual system could, in principle, discriminate among spectra that have broadly similar shapes, but differ slightly in detail. Stomatopods are brightly coloured animals. Some of their markings have species-specific colours, but the hues of others vary considerably among individuals^{21,24}. Recognition of fine spectral detail may play an important part in intraspecific communication and in the recognition of individuals. □

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Accelerated evolution of a false-truffle from a mushroom ancestor

Thomas D. Bruns^{*§}, Robert Fogel[†], Thomas J. White[‡] & Jeffrey D. Palmer[‡]

^{*} Department of Botany, University of California, Berkeley, California 94720, USA

[†] Department of Biology, University of Michigan, Ann Arbor, Michigan 48109–1048, USA

[‡] Cetus Corporation, 1400 Fifty-third Street, Emeryville, California 94608, USA

THE false-truffles (Hymenogastrales) are a group of basidiomycetous fungi that produce underground truffle-like basidiocarps. They are generally believed to be independently derived from several mushroom lineages^{1–4}, but extensive morphological divergence often obscures recognition of these phylogenetic connections. Comparisons of mitochondrial DNA now demonstrate a surprisingly close relationship between species of false-truffles in the genus *Rhizopogon* (Hymenogastraceae) and the mushroom genus *Suillus* (Boletaceae). The striking morphological differences separating all *Suillus* species from *Rhizopogon* imply an acceleration in the rate of morphological change relative to molecular change during the evolution of these false-truffles from their mushroom ancestors. This acceleration can best be explained by rapid morphological divergence resulting from selective pressures

§ To whom correspondence should be addressed.

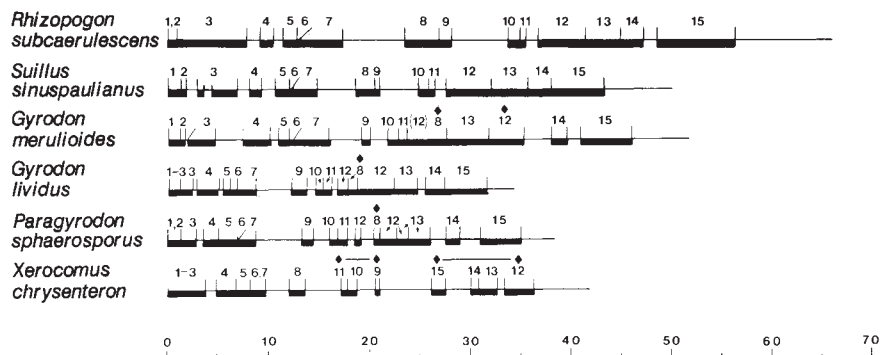
which may have acted on a small number of developmental genes.

Two types of molecular evidence demonstrate that *Rhizopogon* is closely related to *Suillus*. First, the structure of the mitochondrial genome of *Rhizopogon subcaerulescens* is identical to that of 14 species of *Suillus* with respect to the order of 15 regions (Fig. 1). The common order of these fragments in *Suillus* and *R. subcaerulescens* is highly significant because mitochondrial gene order is known to be extremely variable among distantly related species of fungi^{5–7}, and because differences in the order of these 15 regions exist both within the genus *Suillus* and among related genera of the Boletaceae⁸. Second, nucleotide sequences from a portion of the mitochondrial large subunit ribosomal RNA gene from two species *Rhizopogon*, three species of *Suillus* and three species from other genera in the Boletaceae, demonstrate a high level of similarity between *Rhizopogon* and *Suillus* and significant divergence of both of these genera from the other members of Boletaceae sampled (Fig. 2). Cladistic analyses of these sequences demonstrate that *Rhizopogon* and *Suillus* are a monophyletic group that has diverged significantly from the three members of the Boletaceae sampled (Fig. 3).

The extreme morphological divergence between *Rhizopogon* and *Suillus* provides a striking contrast to their molecular similarity. *Suillus* shares many obvious morphological similarities with *Paragyrodon sphaerosporus* such as the presence of tubes, annulate stipes and large pilei. Microscopic features of their basidia and spore attachment are also similar, and the latter species has previously been placed within *Suillus*⁹. *Rhizopogon* differs from both taxa with respect to all of these features and has traditionally been placed in a different family or order^{10–12}, but molecular criteria (Figs 1 and 2) demonstrate that it is more closely related to *Suillus* than either *Suillus* or *Rhizopogon* is to *P. sphaerosporus*. These results demonstrate an accelerated rate of morphological change relative to molecular change during the evolution of *Rhizopogon*.

This acceleration can be caused by either an increase in the rate of morphological evolution or a decrease in the rate of molecular divergence in *Rhizopogon*, relative to the other taxa studied. The latter explanation would require a major departure from the molecular clock hypothesis, and would not explain the structural similarity of the mitochondrial genomes (Fig. 1). The rate of sequence divergence in *Rhizopogon* would have had to have a fourfold decrease just to set its time of divergence from its closest relatives within *Suillus* to that of its nearest mushroom relative outside the genus (that is, *P. sphaerosporus*), and even this adjustment would be insufficient to match the morphological divergence between *Rhizopogon* and *Suillus* to an outside point of reference. Furthermore, this fourfold rate difference clearly represents an underestimation, because four regions of the gene that align well in comparisons of *Suillus* and *Rhizopogon* were excluded from comparisons with the highly divergent sequences of the other three members of the Boletaceae (Fig. 2). Deviations

FIG. 1. Fragment order of *R. subcaerulescens* and five taxa from the Boletaceae. The circular mitochondrial genomes of the six taxa are shown linearized in approximate alignment. Regions that hybridize to 14 cloned fragments of *Suillus* mitochondrial DNA (mtDNA) and to portions of the ATPase 9 gene of *Saccharomyces cerevisiae* (region 8) were determined by Southern blot hybridizations. The fragment order shown in *S. sinuspaulianus* is shared by 14 of the 15 species of *Suillus* previously examined⁸. Rearrangements relative to this predominate *Suillus* order were not found in *R. subcaerulescens* but do exist (diamonds) in all other members of the Boletaceae sampled previously⁸. Approximately 75% of the *S. sinuspaulianus* mitochondrial genome and at least eight mitochondrial genes are included in these fragments⁸. MtDNA size varies threefold within *Suillus*⁷ and is a poor indicator of phylogenetic affinity⁸. Size scale at the bottom is in kilobase pairs. Light hybridization of



clone 12 to a portion of the *G. meruloides* genome is shown with parentheses. The clones, hybridization conditions, and restriction enzymes mapped are described elsewhere⁸.

FIG. 2 Aligned sequences of *Rhizopogon subcaerulescens* (Rs), *R. ochraceorubens* (Ro), *Suillus cavipes* (Sc), *S. luteus* (Sl), *S. sinuspaullianus* (Ss), *Boletus satanas* (Bs), *Xerocomus chrysenteron* (Xc) and *Paragyrodon sphaerosporus* (Ps) from a 351 base-pair (bp) portion of the mitochondrial large subunit rRNA gene. Gaps introduced into the sequence to facilitate the alignments are indicated by stops. Bases that differ from the consensus are underlined. The four large underlined regions highlight areas where the aligned sequences of *Suillus* and *Rhizopogon* species cannot be unambiguously aligned with those of the three other taxa. The sequences start at ~122 nucleotides from primer 1 and end 20 nucleotides from primer 2 (see below). Initial alignments were made with GENALIGN and visually adjusted.

METHODS. A fragment containing the mitochondrial large subunit rRNA gene of *S. sinuspaullianus* was cloned into a bluescript phagemid vector⁸, and subcloned by *ExoIII* deletions²². Single strands of individual clones were rescued²³ with the helper phage M13K07 and sequenced by standard dideoxy-termination method with modified T7 DNA polymerase (Sequenase). Conserved regions of the *Suillus* gene were identified by comparison with published sequences of *Saccharomyces cerevisiae* available in GENBANK. The following two oligonucleotide primers, which are complementary to opposite strands of two conserved regions, were synthesized on a Biosearch 8700 DNA synthesizer: ACCTATGCAGCTTCTACTG, and TTATCCCTAGCGTAACCTTTATC. In the *S. sinuspaullianus* gene these regions are separated by 480 bp (data not shown). *HindIII* fragments containing the large subunit rRNA gene from *S. luteus*, *S. cavipes* and *R. subcaerulescens* were cloned into pUC19 by methods described previously⁸. The two primers were used to sequence the region between them in each of these clones by using CsCl-purified double-stranded plasmids as templates²⁴. The same primers were used in conjunction with the polymerase chain reaction to amplify and sequence the homologous region in the three other members of the Boletaceae and in *R. ochraceorubens*. Double-stranded fragments were amplified in 100- μ l reaction with the following components: 50 pmol each primer, 2.5 units *Taq* polymerase, 0.1–10 ng fungal DNA in a solution of 50 mM KCl, 10 mM Tris, pH 8.4, 2.5 mM MgCl₂, 0.1 mg ml⁻¹ gelatin and

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Rs ACTAAAAATGGGAGAGCTGATTTGATGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
Ro AATAAAAATGGGAGAGCTGATTTGATGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
Sc AATAAAAATGGGAGAGCTGATTTGATGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
S1 ATAGAAATGGGAGAGCTGATTTGATGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
Ss AATAAAAATGGGAGAGCTGATTTGATGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
Bs TTAAGAGGAAATAGCTACATAGTGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
Xc TTAAGAGGAAATAGCTACATAGTGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
Ps TTAAGAGGAGATAGTGGCAGTTGACTGGCAGTTGACTGGGCGGTCGTCTTTAATAGAGTAGCAAAAGTACATCCAAATATAAAATIAAAAT
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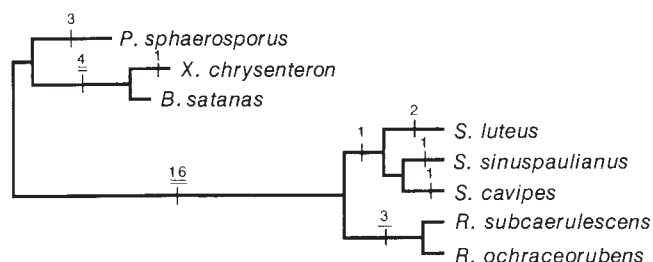
```
Rs .AAAAATATTACTATT.AAATAATATAATGATGATAATTTCTCACAACATCATATTCCTGCTGAAATTTTAAATTTTAACTT
Ro .AAAAATATTACTATT.AAATAATATAATGATGATAATTTCTCACAACATCATATTCCTGCTGAAATTTTAAATTTTAACTT
Sc .AAAAATATTACTATT.AAATAATATAATGATGATAATTTCTCACAACATCATATTCCTGCTGAAATTTTAAATTTTAACTT
S1 .AAAAATATTACTATT.AAATAATATAATGATGATAATTTCTCACAACATCATATTCCTGCTGAAATTTTAAATTTTAACTT
Ss .AAAAATATTACTATT.AAATAATATAATGATGATAATTTCTCACAACATCATATTCCTGCTGAAATTTTAAATTTTAACTT
Bs TAGATAATATTACTATT.TATTTTAAATGATAATATTCGACAAATATTTTAAATTTTAAATTTTAACTT
Xc TAGATAATATTACTATT.TATTTTAAATGATAATATTCGACAAATATTTTAAATTTTAAATTTTAACTT
Ps .AAATCAATATTACTTTTAAAGTAAATATTCGACAAATATTTTAAATTTTAAATTTTAACTT
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Rs TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
Ro TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
Sc TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
S1 TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
Ss TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
Bs TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
Xc TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
Ps TAACATCAATTTTATTTTGTGTTTAAAGGATAGCTAGAAATGAAATGGAGATCAATTAACAGTGAATAGGTTATGGAGGGTGA
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Rs ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
Ro ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
Sc ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
S1 ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
Ss ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
Bs ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
Xc ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
Ps ATGGCGGTAAAGCATGCTTAACTGATAGACTTAAAGTGGCAGATAGCTAAGTAGGGCATAGTGAACCCCTGGTATATTA
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200 μ M each of the four deoxynucleotide triphosphates. These reactions were subjected to 25 cycles in a Perkin-Elmer-Cetus DNA Thermal Cycler under the following temperature regime: 25 s at 95 °C, 55 s at 53 °C, 2 min at 72 °C. Amplified fragments were isolated from a 5- μ l aliquot by gel electrophoresis in 1.5% low-melting agarose. Gel slices containing the fragments were melted into 1 ml of 10 mM Tris, pH 8.0, 1 mM EDTA and diluted 1,000-fold in water. Single-stranded templates of both strands were amplified separately and sequenced directly from 1 μ l the diluted fragment by the asymmetric primer ratio method²⁵. Conditions for these second amplifications were as described above, except that 40 cycles were run with the annealing temperature set at 50 °C and the primer ratios of 1:50 pmol and 50:3 pmol were used to produce strands complementary to the first and second primer respectively.

FIG. 3 Phylogenetic relationships between *Rhizopogon* and members of the Boletaceae based on cladistic analyses of nucleotide sequence differences. The tree is based on parsimony analysis, which minimizes the number of mutational events necessary to account for the sequence differences. It was constructed using the branch and bounds algorithm of the DNAPENNY program²⁶. All site differences analysed were consistent. Thus, this tree postulates no convergence or reversals. Branch lengths correspond to changes within the well-aligned portion of the large subunit rRNA sequence (that is, excluding the four underlined regions; Fig. 2). Branching patterns depicted within the *Suillus*–*Rhizopogon* lineage are based on differences within the entire sequenced region, as these five sequences are aligned throughout. We estimated the confidence in all putatively monophyletic groups larger than one with a bootstrap analysis of sample-size 100 using the DNABOOT program²⁶. Those groups that occurred in 97% or 100% of



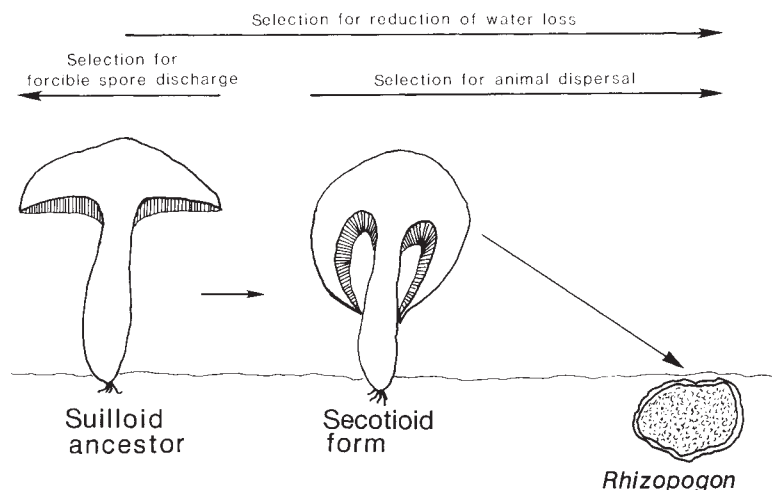
the trees are indicated by branch lengths that are underlined once or twice respectively. The groupings of all *Suillus* species, and of *S. sinuspaullianus* with *S. cavipes* were found in 90 and 69 per cent of trees, respectively.

in the molecular clock have been documented^{13–16}, but differences of this magnitude are generally seen only in comparisons of distantly related lineages¹⁵. No obvious mechanism is available to explain such a drastic rate reduction in this case.

Rapid morphological divergence could be accomplished if the number of underlying genetic changes is small and if selection is intense. A model for the evolution of gastromycetes proposed by Thiers² invokes both of these factors and is applied to *Rhizopogon* in Fig. 4. Unopened, distorted mushrooms, known as secotioid forms, are known in many unrelated lineages and are thought to be caused by developmental arrest during mushroom maturation². In one such case, genetic studies suggest that this arrest may involve only a single recessive gene¹⁷. The presence of secotioid or partially secotioid derivatives of several *Suillus* species shows that a similar process occurs within the

genus^{18–20}. Transition between the secotioid and underground false-truffle forms presumably requires numerous additional mutations. But, many of the genes responsible probably regulate processes related to mushroom development (for example, expansion of the stipe, organization of the hymenium) and changes in such genes might be expected to have pleiotropic effects on the morphology. Furthermore, most morphological differences are losses of ancestral structures; given that there are many ways in which gene function can be lost, this process might be expected to be more rapid than one that requires extensive acquisition of novel morphological structures. The enclosed nature of spore-producing tissues in secotioid mushrooms might also be expected to initially increase the frequency of inbreeding, because large clumps of spores would generally be dispersed from a single basidiocarp. A high frequency of

FIG. 4 Model for the evolution of *Rhizopogon* adapted from Thiers². Fruit bodies of a suilloid ancestor, a secotioid form, and *Rhizopogon* are shown in cross-section. Spore-bearing regions are shown with lines (that is, tubes in the suilloid and secotioid forms) and stippling (in *Rhizopogon*). The initial transition between suilloid mushroom and a secotioid form is thought to involve the arrest of normal pileus expansion. Further modification, by loss or reduction of ancestral features, results from continued selection for reduction of water loss and selection for rodent dispersal of fruitbodies²⁷. The establishment of the initial secotioid form also removes selection for the maintenance of forcible spore discharge, which in turn permits the loss of precisely aligned tubes, loss of spore asymmetry, and the formation of aberrant basidia. Other changes which occur during the transition to the *Rhizopogon* form are the reduction and loss of the stipe, size reduction of the basidiocarp, loss of cystidia, loss of sterigmata, and completion of development underground.



inbreeding would enable a rapid increase in the frequency of recessive alleles in the progeny.

The sparsity of intermediates between *Rhizopogon* and *Suillus* provides indirect evidence of intense selective pressure. Putatively intermediate taxa are limited to three secotioid forms of *Suillus* and two to three species of *Truncocolumella*. Collectively, these fungi do not approach a gradual set of intermediates. With the exception of *Truncocolumella citrina*, all are rare and limited in distribution. In contrast, both *Suillus* and *Rhizopogon* have over 80 described species, many of which are common and widely distributed in north temperate regions. This pattern is consistent with the concept of a selective gradient in which intermediates, ill-adapted to either air or animal dispersal and lacking the derived modifications necessary for seasonally xeric conditions, would be eliminated at a high frequency.

The dramatic change in morphology and lack of intermediates seen in these extant organisms is analogous to the patterns seen in the fossil records of many other taxa²¹. The case of *Suillus* and *Rhizopogon* thus provides a modern example of rapid morphological evolution; one in which a detailed proposed mechanism exists, and where the underlying molecular genetic basis can be examined in more detail. □

Origin of the algae

Roland Perasso, Anne Baroin, Liang Hu Qu*, Jean Pierre Bachelier* & André Adoutte

Laboratoire de Biologie Cellulaire 4 (URA 25 CNRS), Batiment 444, Université Paris Sud, 91405 Orsay Cedex, France

* Centre de Recherche de Biochimie et Génétique Cellulaires (LP 8201 CNRS), 118 route de Narbonne, 31062 Toulouse Cedex, France

EUKARYOTIC algae are traditionally separated into three broad divisions: the rhodophytes, the chromophytes and the chlorophytes. The evolutionary relationships between these groups, their links with other eukaryotes and with other photosynthetic groups, such as euglenophytes and cryptophytes, have been the subject of much debate and speculation¹⁻⁸. Here we analyse partial sequences of the large (28S) cytoplasmic ribosomal RNA from ten new species of protists belonging to various groups of unicellular algae. By combining them with the homologous sequences from 14 other unicellular and multicellular eukaryotes, we show that rhodophytes, chromophytes and chlorophytes emerge as three distinct groups late among eukaryotes, that is, close to the metazoa-metaphytes radiation. This implies a relatively late occurrence of eukaryotic photosynthetic symbiosis. We also provide details of intra- and inter-phyla relationships.

The ten new sequences, as shown in Fig. 1, correspond to the ~450 nucleotides of the 5' end of the cytoplasmic large ribosomal RNA (28S). This molecule can be used as a tracer of the history of the nucleo-cytoplasmic compartment independently of the chloroplast compartment, which is now widely considered to result from a single or from several endosymbiotic events⁹⁻¹². We have shown that the specific domain sequenced provides a good phylogenetic index over very broad evolutionary distances^{13,14}. The phylogenetic relationships of algae with the eukaryotic kingdoms (metazoa, metaphytes, fungi and protists) show that all the algal groups emerge later than all other unicellular eukaryotes (Fig. 2). Both Sogin's group¹⁵ and ours¹³ have previously reported that a set of non-photosynthetic flagellated protists emerge very early from the eukaryotic tree. It now appears that the three main classes of photosynthetic protists as well as the cryptophytes diverged at a later stage (as predicted by Cavalier-Smith¹⁶ on the basis of several ultrastructural features), during a period of intense diversification that is relatively close to the metazoa-metaphytes radiation and which comprises the fungi and other groups of protists such as ciliates. This rapid diversification makes it difficult to resolve branching orders in distance trees. However, several alternative procedures for treating the data, including parsimony approaches

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(Swofford's PAUP and Felsenstein DNA parsimony programs), yield similar topologies (data not shown). We favour the view that there was a relatively long period of non-photosynthetic eukaryotic protists (see also ref. 17) followed by an explosive period closely predating the radiation of plants and animals. This may correspond in chronological terms to the late Precambrian.

With all types of tree-construction methods used (distance and parsimony), we observe a deep separation between chromophytes, chlorophytes and rhodophytes which fits quite well with classical algae systematics. Comparative analysis of

these sequences within our limited number of photosynthetic representatives opens in each group the possibility of an independent confrontation with classical systematics.

First, the chlorophytes, as expected and already confirmed by Sogin's group¹⁸ and by ours¹⁹, share a direct common ancestor with land plants. In addition, we observe that *Chlorogonium elongatum* (a Chlorophyceae) always diverges from the chlorophyte lineage more deeply than *Pyramimonas parkeae* (a Prasinophyceae). This is compatible with some schemes.²⁰

Second, the chromophytes, Chrysophyceae (*Ochromonas danica* and *Synura petersenii*), Rhaphidophyceae (*Vacuolaria*

M. musculus	CGGACCCU	GAUCAGAC	CG	UGGCGACCG	CUGAAUUUA	GCAGAUUUAU	CAGGCGAGGA	AAAGAAACUA	ACGAGGAUUC	CCUCAGU*AA	CGGCGAGUGA	ACAGGGA*AG	AGCCGAGGSC	C*GAUCCGCC	GGCGGCGUC	GCGGCGUGG	146
X. laevis	UCA	U		C			C	A				G			CG	C	143
O. sativa		C	G	G	A	U		G		C		C	G	A	U	G	142
Z. mize	XX	C	G	U		G	A	A		C		C	G	C	U		139
N. tabacum	XX	C	G		A	U		G		C		C	C	A	C	U	142
→ C. elongatum	XCA	C	G	C	A	C		C		C		C	C	A	U	G	136
→ P. parkeae	XIU	U		C	A	A		C		C		C	C	A	U	G	139
→ V. virescens	XCA	C	U	A	A	A		C		C		C	C	A	U	G	146
→ C. roscoffensis	XXXXC	A	G	C	A	A		C		C		C	C	A	U	G	139
→ P. parvum	XXXXC	A	C		A	A		C		C		C	C	A	U	G	139
→ S. petersenii	XCG	C	A	U	A	A		C		C		C	C	A	U	G	130
→ O. danica	XXX	C	A	U	A	A		C		C		C	C	A	U	G	127
→ P. purpureum	XX		G	A	A	U		C		C		C	C	A	U	G	142
→ C. parametrium	XXXXXX	A	G	A	A	U		C		C		C	C	A	U	G	129
→ C. ovata	XXU	U	A	G	A	U		C		C		C	C	A	U	G	128
S. oerevisiae	XUU	A	G	A	A	U		C		C		C	C	A	U	G	142
N. crassa	XUU	A	G	A	A	U		C		C		C	C	A	U	G	137
F. oxysporum	XUU	A	G	A	A	U		C		C		C	C	A	U	G	139
P. primaurelia	XCAC	G	A	A	A	U		C		C		C	C	A	U	G	142
B. japonicum	XAGU	G	A	A	A	U		C		C		C	C	A	U	G	132
S. oerevisiae	XAGC	G	A	A	A	U		C		C		C	C	A	U	G	132
T. vaginalis	XXUAGU	GC	C	A	A	A		C		C		C	C	A	U	G	133
C. fasciculata	XCA	A	G	A	A	U		C		C		C	C	A	U	G	142
D. discoideum	U	C	G	A	A	U		C		C		C	C	A	U	G	142
E. coli	U	A	A	A	A	U		C		C		C	C	A	U	G	127

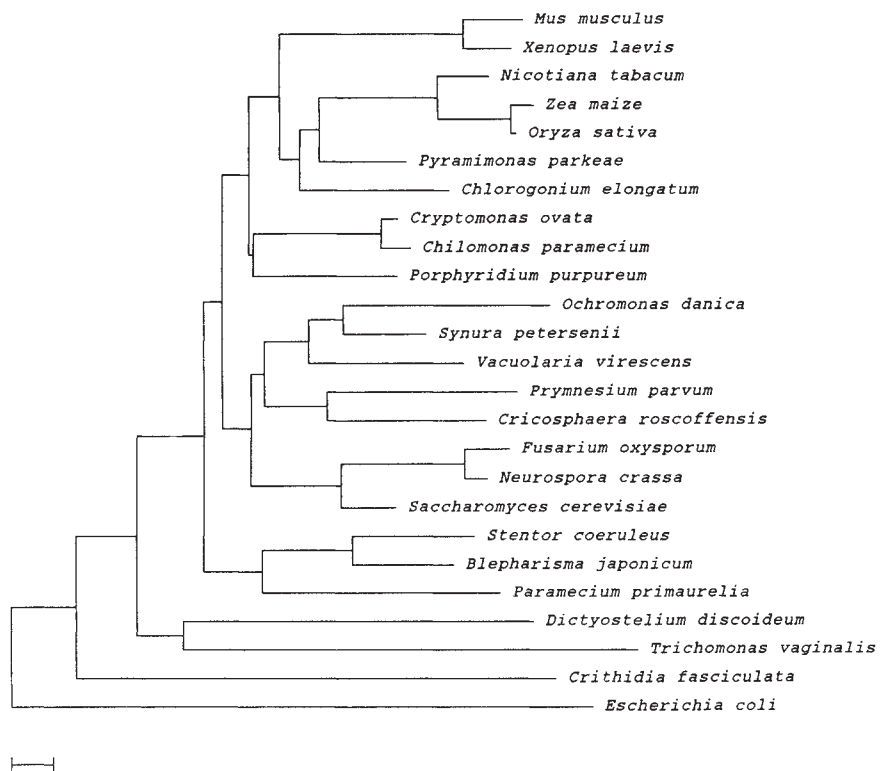
M. musculus	AAUUGUGG	UACGAGAC	CC	*ACUC	C	CGGCGCGC	UGG	*****	*UGGGGCGC	CAUUGC	UU	*CUGAUC	AGGCC	*****	AGGCCGUG	*****	GA	CGGUGAGG	CGGUGAGG	CGGCCG	*****	GGCGCGCG	261
X. laevis	CG			G	G	C	C																258
O. sativa	U	A	U	C	G	A	G																248
Z. mize	U	A	U	C	G	A	G																245
N. tabacum	U	A	U	C	G	A	G																247
→ C. elongatum	U	A	U	C	G	A	G																242
→ P. parkeae	U	A	U	C	G	A	G																246
→ V. virescens	U	A	U	C	G	A	G																253
→ C. roscoffensis	U	A	U	C	G	A	G																244
→ P. parvum	U	A	U	C	G	A	G																237
→ S. petersenii	U	A	U	C	G	A	G																242
→ O. danica	GG	A	U	C	G	A	G																234
→ P. purpureum	G	A	U	C	G	A	G																248
→ C. parametrium	U	A	U	C	G	A	G																239
→ C. ovata	U	A	U	C	G	A	G																237
S. oerevisiae	GU	A	U	C	G	A	G																241
N. crassa	GU	A	U	C	G	A	G																249
F. oxysporum	GU	A	U	C	G	A	G																243
P. primaurelia	U	A	U	C	G	A	G																249
B. japonicum	GU	A	U	C	G	A	G																236
S. oerevisiae	U	A	U	C	G	A	G																236
T. vaginalis	U	A	U	C	G	A	G																226
C. fasciculata	GU	A	U	C	G	A	G																287
D. discoideum	UG	A	U	C	G	A	G																252
E. coli	*****	*GUG	GU	*G	*G	*G	U																196

M. musculus	GGCUGGUC	UUGCGGAGU	CGGUGGUCU	**GGGAGU	CAGGCCAAG	C*GGGUGUA	ANUCUCCAU	AAGGCUAAU	ACC**GGAC	GAGACGAAU	GUCACAGU	ACCGUAGG	AAUGU	380
X. laevis	AC	C	U											376
O. sativa	ACGAG	C	C											367
Z. mize	ACGAG	C	C											364
N. tabacum	ACGAG	C	C											366
→ C. elongatum	ACGAG	C	C											361
→ P. parkeae	ACGAG	C	C											365
→ V. virescens	ACGAG	C	C											362
→ C. roscoffensis	ACGAG	C	C											363
→ P. parvum	ACGAG	C	C											361
→ S. petersenii	ACGAG	C	C											356
→ O. danica	ACGAG	C	C											352
→ P. purpureum	ACGAG	C	C											367
→ C. parametrium	ACGAG	C	C											358
→ C. ovata	ACGAG	C	C											356
S. oerevisiae	UAAA	U	C											368
N. crassa	UAAA	U	C											360
F. oxysporum	UAAA	U	C											362
P. primaurelia	CC**GAA	G	A											366
B. japonicum	AGGUCC	G	A											355
S. oerevisiae	AA	*GACU	G	A										354
T. vaginalis	AA	*GACG	G	A										341
C. fasciculata	CUGAACU	A	A											409
D. discoideum	UUGC	AUGU	A	A										371
E. coli	AUGCU	U	A	G										317

FIG. 1 Sequence alignments for the 5'-terminal region of nuclear-encoded large-subunit rRNA. Arrow, the photosynthetic eukaryotic species that we have sequenced in this study. Direct sequencing of the RNA was performed as described in ref. 13. For sequence data of species not described in this paper see refs 13 and 24. Only the nucleotides that differ from those of the mouse are shown (identities denoted by hyphens, deletions by stars).

The nucleotide positions that could not be identified are denoted by X. This portion of the molecule contains conservative domains (overlined) in which substitutions do not reach saturation levels while allowing meaningful calculations. Rapidly evolving areas which are suitable for comparison of more closely related species are excluded from the present analysis.

FIG. 2. Phylogenetic tree constructed using a least-square distance method (the 'Fitch' program of the Felsenstein-Phylip package with the G global-rearrangement option). Pairwise comparisons of the rRNA sequences have been carried out over regions overlined in Fig. 1. *Escherichia coli* serves as an outgroup. Computation of the corrected number of nucleotide differences (using Kimura's K_{nuc} correction) and derivation of the resulting distance-matrix were carried out exactly as described¹⁴. In this tree, the distance between two species is proportional to the sum of the projections on the x (K_{nuc}) axis of the branch lengths, whereas distances on the y axis are arbitrary. 2,481 trees were examined and the average per cent standard deviation was 7.14. Scale bar, 0.019 units K_{nuc} .



virescens), Haptophyceae (*Prymnesium parvum* and *Cricosphaera roscoffensis*) and fungi (*Saccharomyces cerevisiae*, *Neurospora crassa* and *Fusarium oxysporum*) are associated into a single, well-individualized branch. The deep demarcation observed between the Haptophyceae and the Chrysophyceae was expected because Haptophyceae are the most remote in structure from the other chromophytes²¹. Conversely, the relative closeness of the Rhaphidophyceae and the Chrysophyceae is also in agreement with most structural and biochemical evidence. Concerning the fungi, whose origin is still debated, it has often been proposed in the past and again more recently²², that they derive from a red algal ancestor. Although we have sequenced only a single red algae, our data do not support such a view at the moment. In all our trees, fungi emerge systematically within the chromophyte branch, albeit deeply rooted. Gunderson *et al.*¹⁸, on the basis of complete 18S rRNA sequences, found an oomycete (*Achlya bisexualis*) in close association with a unique representative of chromophytes (*O. danica*). As for Eufungi, the three species they analysed (*S. cerevisiae*, *N. crassa* and *Podospira anserina*) emerged immediately after this oomycete-chromophyte pair, but on an independent branch. Because Gunderson *et al.*¹⁸ used larger sequences than us, their topology may be more significant statistically than ours—Eufungi and chromophytes may not share a common ancestor. At any rate, a close relationship exists between fungi and chromophytes, suggesting that they appeared roughly at the same time.

Third, the unique representative of rhodophytes we have sequenced (*Porphyridium purpureum*) branches off independently from chlorophytes and chromophytes as a distinctive algal group. We note that Cryptophyceae (*Cryptomonas ovata* and *Chilomonas paramecium*) form a sister group of *Porphyridium purpureum*. More data from red algae are necessary to settle their precise phylogenetic relatedness with other algae.

This analysis of nuclear-encoded rRNA shows that algae do not form a coherent and unique group relative to non-photosynthetic ones. Indeed, in none of our trees did a clear filiation between the various algal divisions emerge. Rather, three distinct, possibly monophyletic ensembles were systematically

observed. This can be fitted with the notion of multiple independent symbiotic events at the origin of photosynthetic protists. However, the alternative hypothesis of the single symbiotic origin theory cannot be excluded by our dendrograms. In this latter case, the possibility of secondary loss of chloroplasts in metazoa, as well as a strong diversification of this organelle during a very short period of time, has to be admitted. Discrimination between these alternatives may emerge from analysis of the phylogeny of chloroplast genome itself²³. In any case, these models of symbiotic events appear to have occurred at an advanced stage of protistan diversification and hence much later than for the mitochondrial counterpart. □

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Phylogenetic analysis based on rRNA sequences supports the archaeobacterial rather than the eocyte tree

Manolo Gouy*† & Wen-Hsiung Li*‡

* Center for Demographic and Population Genetics, University of Texas, PO Box 20334, Houston, Texas 77225, USA

† Laboratoire de Biométrie, Université Lyon I, 69622 Villeurbanne Cedex, France

How many primary lineages of life exist and what are their evolutionary relationships? These are fundamental but highly controversial issues¹. Woese and co-workers²⁻⁴ propose that archaeobacteria, eubacteria and eukaryotes are the three primary lines of descent and their relationships can be represented by Fig. 1a (the 'archaeobacterial tree') if one neglects the root of the tree. In contrast, Lake^{5,6} claims that archaeobacteria are paraphyletic, and he groups eocytes (extremely thermophilic, sulphur-dependent bacteria) with eukaryotes, and halobacteria with eubacteria (the 'eocyte tree', Fig. 1b). Lake's view has gained considerable support as a result of an analysis⁶ of small subunit ribosomal RNA sequence data by a new approach, the evolutionary parsimony method⁷. Here we report that analysis of small subunit data by the neighbour-joining and maximum parsimony methods^{8,9} favours the archaeobacterial tree and that computer simulations using either the archaeobacterial or the eocyte tree as a model tree show that the probability of recovering the model tree is very high (>90 per cent) for both the neighbour-joining and maximum parsimony methods but is relatively low for the evolutionary parsimony method. Moreover, analysis of large subunit rRNA sequences by all three methods strongly favours the archaeobacterial tree.

We use species (see Fig. 1c) for which both the small and large subunit (SSU and LSU respectively) rRNA sequences are available so that the congruency of the SSU and LSU trees inferred by a method can be checked; more species than in Fig. 1c can be included, but this does not change our conclusion. SSU rRNAs are aligned as published¹⁰ but only those regions (876 sites) that have been well conserved are used in our analysis. LSU rRNAs are aligned so that they fit the consensus secondary structure folding model¹¹: only 1,658 sites are used. We shall consider other alignments below.

We first show that analysis of LSU data by the evolutionary parsimony (EP) method strongly contradicts Lake's conclusion⁶ based on an application of the same method to SSU data. The EP method applies only to four lines of descent, although each line may contain multiple species. For each of the three possible unrooted trees, a χ^2 with one degree of freedom is calculated from the nucleotide configurations among the four lineages, and a tree is taken to be the true tree if the associated χ^2 is significant, whereas the other two values of χ^2 are not. Application of this method to the SSU data from the eukaryotes, eubacteria, eocytes and halobacteria given in Fig. 1c gives the χ^2 values of 3.5, 3.6 and 1.5, respectively, for the archaeobacterial, the eocyte and the halobacterial tree (halobacteria-eukaryotes, eocytes-eubacteria). None of these χ^2 values is significant, but if more eukaryotic and eubacterial species are included, the eocyte tree is strongly favoured, in agreement with Lake's conclusion⁶. But when the EP method is applied to the LSU data, the three χ^2 values become 29.5, 0.4 and 0.3, strongly supporting the archaeobacterial tree ($P < 5 \times 10^{-6}$). When the SSU and LSU data are combined, the three χ^2 values are 13.0, 3.1 and 2.6, and the archaeobacterial tree is again strongly favoured ($P = 0.0003$). (Actually, the significance level should be about $3P$ instead of P , because three

tests are made.) If the *Methanococcus* SSU and LSU rRNA sequences are also included in the analysis and are placed with the halobacterial lineage (Fig. 1c), the χ^2 values are 27.3, 5.1 and 2.3, supporting even more strongly the archaeobacterial tree. Further, it is not tenable to place *Methanococcus* in either the eukaryote or the eubacterial lineage, because in both cases the EP method fails to find a unique statistically significant χ^2 value.

Next, we analyse the data by the neighbour-joining (NJ) and maximum parsimony (MP) methods, which are known to be efficient for inferring the true tree. The NJ algorithm is to sequentially find neighbours (sequence pairs) that minimize the total branch length of the tree. Application of this method to the SSU and LSU data either separately or jointly gives the same tree topology shown in Fig. 1c. The same is true for the MP method; for the SSU data, the archaeobacterial, eocyte and halobacterial trees are supported by 20, 10 and 9 informative sites, respectively, and for the LSU data, the corresponding numbers are 34, 27 and 14. Thus, complete congruency is observed, regardless of which of the two methods and which data set are used.

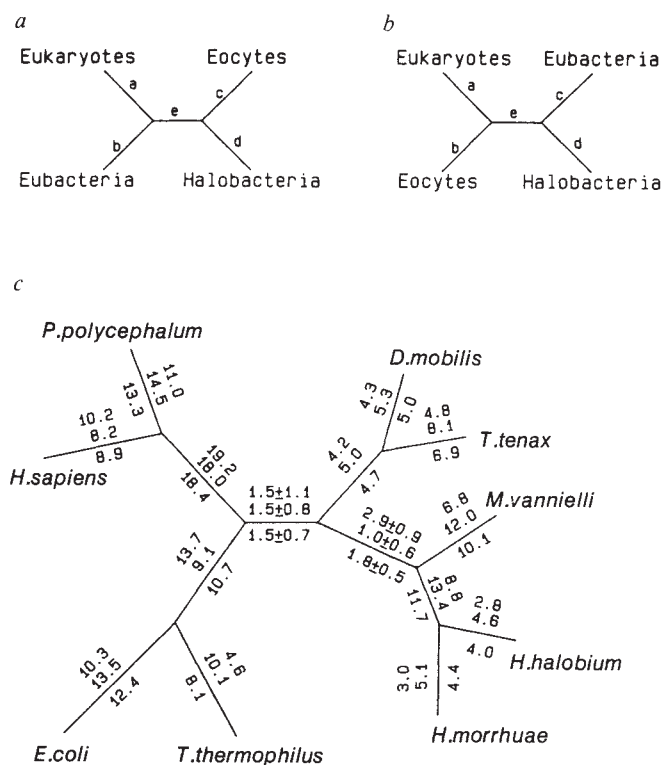


FIG. 1 a, The archaeobacterial tree; for simplicity, methanogens are not included. b, The eocyte tree. c, The unrooted tree inferred by the neighbour-joining and the maximum parsimony methods^{8,9}. The branch lengths were estimated by the least-squares method after the topology was determined; the number of nucleotide substitutions between sequences was estimated by the two-parameter method²⁴, which allows for unequal rates between transitional and transversal substitutions. The first, second and third (below the branch) values on each branch represent the estimated numbers of substitutions per 100 sites for the SSU data, the LSU data (including, for eukaryotes, the 5.8S rRNA) and the combined data, respectively. The standard errors (s.e.) were obtained by the method in ref. 25; a branch length is significantly greater than 0 at the 5% level if the mean is two times the s.e. or larger. The species used are: *Homo sapiens*^{10,14} and *Physarum polycephalum*^{14,26} (eukaryotes), *Thermus thermophilus*^{27,28} and *Escherichia coli*^{10,14} (eubacteria), *Thermoproteus tenax*^{10,14} and *Desulfurococcus mobilis*^{10,14} (eocytes), *Halobacterium halobium*^{10,14} and *Halococcus morrhuae*^{10,14} (halobacteria), and *Methanococcus vannielli*^{10,14} (methanogens). For eukaryotes, the two species used are the two most divergent species for which both the SSU and LSU rRNA sequences are available; this is also the case for eubacteria. The alignments used are available upon request.

‡ To whom correspondence should be addressed.

Having determined the topology, we calculate the branch lengths of tree 1c by the least-squares method. For the archaeobacterial group, the branch lengths estimated from the LSU data tend to be longer than those estimated from the SSU data, whereas no clear tendency is observed for the eukaryotic and eubacterial groups. Thus, the problem of unequal rates among the four groups is less pronounced for the LSU data than for the SSU data. For the internal branch connecting the central node to the archaeobacteria, the mean is more than twice the standard error of the estimated branch length when the SSU and LSU data are combined, and so the separation of the archaeobacteria from the other two groups is statistically significant. The statistical significance is also supported by bootstrapping¹² either the SSU or LSU data with either the NJ or MP method (ref. 13; our result). On the other hand, if the eocytes are clustered with the eukaryotes, then the length of the central branch estimated by the least-squares method becomes negative, whether the SSU or the LSU data are used. This result suggests that the eocyte tree is erroneous.

We believe that our alignment using the consensus secondary structure as a reference is superior to Lake's star alignment⁶. In the latter, the eocyte *Thermoproteus tenax* sequence was taken as the standard, and a representative from each of the other groups was aligned pairwise against this sequence. This approach implicitly assumes that the *T. tenax* sequence is the ancestral sequence and makes no attempt to achieve a global optimum because in aligning a sequence against the *T. tenax* sequence no reference is made to any other sequences. On the other hand, the consensus LSU rRNA secondary structure has been thoroughly studied by many authors^{11,14} in an attempt to find a folding model that best fits to all available sequences. Of course, there are some regions that do not have homologues in all species and others in which the consensus structure is not certain, but most of these regions are excluded in our analysis; we use only regions that involve few or no gaps and can be unambiguously aligned. In contrast, Lake also included regions where alignment is very uncertain because many gaps are involved or because many substitutions have occurred (he used 984 sites, whereas we use only 876). At any rate, to show that our conclusion is not dependent on the alignment used, we have considered two other alignments for the LSU sequences. We used a multiple alignment algorithm¹⁵ with a score of 2 for a transition, 3 for a transversion, and $7+3n$ for a gap, where n is the number of nucleotides in the gap. We first obtained an alignment for each of the four groups: eocytes, eukaryotes, eubacteria and halobacteria. The four alignments were then aligned in two different orders: (1) eocytes, eukaryotes, halobacteria and eubacteria, and (2) eocytes, eukaryotes, eubacteria and halobacteria. In both alignments, the eocyte tree is assumed

to be the true tree because eocytes and eukaryotes are grouped together first. Nevertheless, even with these alignments all three tree-making methods favour the archaeobacterial tree rather than the eocyte tree.

Lake⁶ has argued that, compared with EP, NJ and MP are more sensitive to effects of unequal rates of nucleotide substitution among lineages. But this is true only when the degree of sequence divergence is large¹⁶. For the SSU and LSU data used in this study the degree of divergence is moderate (about 50% or less), and a computer simulation shows that in this situation NJ and MP both perform better than EP, whether the archaeobacterial or the eocyte tree is used as the model tree (Table 1). The inferiority of the EP method becomes even more conspicuous if the rate of nucleotide substitution is not uniform but follows a log-normal distribution¹⁷ along the sequence (Table 1). In fact, the probability for EP to recover the archaeobacterial tree is as low as 0.41.

The reliability of the three tree-making methods can also be tested empirically by applying each method to the SSU sequences from human, *Drosophila*¹⁸, rice and *Physarum*; 1,110 sites are used. We choose these sequences because the *Drosophila* sequence has evolved faster than the human sequence; the branch lengths for the two lineages are 6.6% and 4.6%. Both NJ and MP give the correct tree, whereas EP favours the tree with human and rice as a sister group ($\chi^2 = 4.8$; the χ^2 values for the correct tree and the tree with humans and *Physarum* as a sister group are 0.6 and 0.3). This branching error is rather serious because, in reality, humans are much more closely related to *Drosophila* than to rice. The EP method has been shown to be sensitive to deviations from the assumption of equal transversional rates (Jin and M. Nei, unpublished results) and thus is likely to be sensitive to changes in G+C content. The G+C contents have diverged substantially among the *Drosophila* (45% for the regions used), human (50%), rice (48%) and *Physarum* (48%) sequences. The eocytes are much richer in G+C (about 64% for both SSU and LSU sequences) than the other groups (ranging from 49 to 60%). Lake⁶ stated that his analysis of SSU data by the EP method was probably not much biased by this large variation in G+C content because two of the three χ^2 values were small. Two of the above χ^2 values are also small, however, but the inferred branching order is erroneous. Further, in our analysis of LSU data, two of the χ^2 values are also small (0.4 and 0.3, see above), yet our result is contradictory to his. Thus, the effects of G+C content may not be detected by the χ^2 analysis. No definite conclusion can be drawn from application of the EP method to the SSU and LSU data.

The eocyte tree does not seem tenable. It is favoured only when the EP method is applied to the SSU data. When this

TABLE 1 Probability of recovering the model tree

Model tree	Branch lengths (%)					EP	Methods	
	a	b	c	d	e		NJ	MP
Uniform rate along the sequence								
Archaeobacterial tree (Fig. 1a)	30.0	21.5	14.4	8.8	1.5	0.50 (0.64)	0.97 (0.98)	0.91 (0.92)
Eocyte tree (Fig. 1b)	23.1	3.8	21.6	13.1	3.8	0.79 (0.91)	0.99 (0.98)	0.99 (0.98)
Log-normal distribution of substitution rates								
Archaeobacterial tree (Fig. 1a)	30.0	21.5	14.4	8.8	1.5	0.41 (0.46)	0.92 (0.94)	0.85 (0.85)
Eocyte tree (Fig. 1b)	23.1	3.8	21.6	13.1	3.8	0.66 (0.78)	0.93 (0.92)	0.95 (0.94)

In all cases 1,000 replicates were conducted and the number of nucleotide sites used was 900, which is about the number of sites used in the case of SSU rRNA sequences. For the model tree shown in Fig. 1a, the lengths of branches a, b, c, d and e are the average values of the branch lengths in Fig. 1c calculated from the SSU data. For the model tree Fig. 1b, the branch lengths were estimated by the operator metrics method²³, which gives the uncorrected number of transversional differences in each branch, from which we estimated the total number of substitutions by the two-parameter method²⁴. We then simulated nucleotide changes in each branch according to the two-parameter model. We assumed a proportion of 55% for transitions, an approximate value estimated from the SSU data; we have also used a proportion of 45% but the results were essentially the same as those in the table. The width of the log-normal distribution was set such that 95% of the sites have a rate between one-eighth and eight times the median rate for any given lineage¹⁷. The values in parentheses are the probabilities of obtaining the correct tree among replicates in which one and only one of the three χ^2 values calculated by the EP method is greater than 3.841 (5% significance level). The EP method uses only a rather small part (transversional differences only) of the sequence information and thus has a relatively low probability of recovering the model tree.

method is applied to the LSU data, the archaeobacterial tree is strongly favoured. This strong incongruency, the computer simulation, and the empirical test all indicate that the EP method is rather inefficient for phylogenetic reconstruction in this situation. By contrast, for the data under consideration, the NJ and MP methods appear to be highly efficient because they have a greater than 90% probability of recovering the model tree (Table 1). Because both methods support the archaeobacterial tree regardless of whether the SSU or the LSU data are used, and because this tree has also been supported by other analyses of rRNA sequence data^{4,11,13,17}, we can conclude with confidence that it is the true tree. But this tree does not explain some molecular properties shared by eubacteria and halobacteria¹⁹.

Currently there is much debate about the root of the tree. One view is that the archaeobacteria represent the urkingdom from which the eubacteria and the eukaryotes have arisen independently, the former lineage from within the sulphur-dependent archaeobacteria (eocytes) and the latter from the methanogen halophile group of archaeobacteria³. (Lake's⁶ view is similar to this, except he does not assume that the archaeobacteria represent the urkingdom.) This view is obviously not compatible with the branching order shown in Fig. 1c. Another possibility^{3,4} is that the root lies in the central branch of Fig. 1c. Both this view and the view⁴ that the eukaryotic, eubacterial and archaeobacterial lines were all derived from a progenote imply that the eukaryotic lineage is as old as the eubacterial lineage, but eukaryotes probably did not appear before 2,000 million years (Myr) ago, whereas eubacteria go back at least 3,500 Myr (ref. 20). A more plausible hypothesis is that the archaeobacteria and the eukaryotes are sister groups because they share marked molecular and cellular resemblances²¹. For example, the archaeobacterial genes coding for the RNA polymerase core subunits are much more similar to the eukaryotic than to the eubacterial counterparts²². Under this hypothesis the eocytes branched off shortly after the divergence between archaeobacteria and eukaryotes, and *Methanococcus* branched off shortly after that. This supports the suggestion that the archaeobacteria form a monophyletic but highly diversified group²⁻⁴. The greater lengths of the halobacterial and methanogen branches compared with that of the eocytic branch may explain why the resemblance between archaeobacteria and eukaryotes is most pronounced in the case of the sulphur-dependent archaeobacteria^{3,6,19}. □

A family of mitochondrial proteins involved in bioenergetics and biogenesis

Ulrich Schulte*, Michael Arretz†, Helmut Schneider†, Maximilian Tropschug†, Elmar Wachter†, Walter Neupert† & Hanns Weiss*

* Institut für Biochemie, Universität Düsseldorf, Düsseldorf, FRG

† Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie, Universität München, Goethestrasse 33, 8000 München, FRG

THE respiratory chain complexes of mitochondria consist of many different subunits, of which only a few partake directly in electron transport. The functions of the subunits that do not contain prosthetic groups are largely unknown¹. The cytochrome reductase complex of *Neurospora crassa*, for example, consists of nine different subunits², of which the peripheral membrane proteins I and II (ref. 3) that are located on the matrix side of the mitochondrial inner membrane⁴ are the largest subunits devoid of redox centres. Significantly, a cytochrome reductase fraction lacking these two subunits was inactive in electron transfer⁵, and in yeast mutants with defective genes for either of the two subunits, assembly of the reductase is disrupted^{6,7}. Most mitochondrial proteins are imported into the mitochondrion as precursor proteins, and two proteins are necessary for cleaving their presequences⁸, namely the matrix processing peptidase (MPP) and the processing enhancing protein (PEP), the latter strongly stimulating the activity of the former⁹. Temperature-sensitive yeast mutants, which are affected in PEP or MPP, accumulate precursors at the non-permissive temperature¹⁰⁻¹². We report here that subunit I of the cytochrome reductase complex of *N. crassa* is identical to PEP and that the protein is therefore bifunctional, participating in both electron transport and protein processing. The processing proteins and subunits I and II of cytochrome reductase can be grouped as members of the same protein family.

We isolated cytochrome reductase as a monodisperse protein-detergent complex¹³ and by treating it with salt cleaved it into three distinct parts, one of which was a subcomplex of subunits I and II⁴. Following purification by sucrose gradient centrifugation⁵, we dissociated the subcomplex by raising the pH and separated the single subunits by DEAE-chromatography and gel permeation chromatography. Pure subunit I was obtained with a yield of ~30%. We raised antibodies against the subunit¹⁴ and used them in conjunction with hybrid-release translation to isolate positive clones from a *N. crassa* complementary DNA library¹⁵. Further screening by colony-filter hybridization was performed with the same library and in a cDNA-library in λ gt11 (ref. 16). Surprisingly, we found that the cDNA sequence was identical to the sequence of the processing enhancing protein⁹. The amino-acid sequence deduced from the cDNA was verified by solid-phase sequencing of isolated subunit I and bromocyanogen peptides thereof (data not shown).

PEP and MPP have been isolated from the mitochondria of both *N. crassa*⁹ and yeast¹⁷. MPP is a soluble matrix protein, accounting for ~0.03% of total mitochondrial protein, whereas PEP comprises 0.5% of total protein and is found in the matrix (~25%) as well as associated with the membranes (~75%) (ref. 9). We compared the abilities of subunits I and II to stimulate the processing activity with that of PEP itself. For the assay, a PEP-free MPP preparation was used to process the precursor of the β -subunit of ATP-synthase ($F_1\beta$). MPP alone showed low processing activity which was stimulated by either isolated PEP, isolated subunit I or whole cytochrome reductase. Subunit II, however, was inactive (Fig. 1). Immunoblotting revealed that antibodies to *N. crassa* subunit I, *N. crassa* PEP, and yeast PEP recognized *N. crassa* subunit I as well as *N. crassa* PEP (Fig. 2a).

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To determine the number of homologous genes, we hybridized restricted genomic DNA of *N. crassa* with PEP/subunit I cDNA (Fig. 2b). The Southern blots showed only bands corresponding to the number of restriction sites expected from the cDNA sequence and from restriction patterns of genomic clones (unpublished data). Hybridization at lower stringencies showed increased background but no additional bands, indicating the absence of a cross-hybridizing DNA and the existence of a single gene for subunit I/PEP.

An alignment of the amino-acid sequences of MPP, PEP and subunits I and II all of yeast, and subunit I/PEP of *N. crassa* (Fig. 3a) reveals significant similarities among the sequences. Whenever one member of this family is compared with the other four members, *N. crassa* subunit I/PEP shows the highest degree of sequence identity, whereas yeast subunit II shows the lowest. The sequence of *N. crassa* MPP (unpublished) is also consistent with this alignment.

Although the evolutionary relationships of cytochrome reductase subunit I and II, PEP and MPP remain enigmatic, it is conceivable that the proteins are relics from a processing protease in the endosymbiotic prokaryotic ancestor of mitochondria. Alternatively, the processing activity may have evolved

from a protein that originally stabilized or facilitated the assembly of multi-protein complexes.

In yeast, subunit I, the *cor1* gene product⁶ and PEP, the *mas1* gene product¹¹, are homologous but not identical. This prompted us to consider the possibility that the enhancement of processing associated with subunit I was the result of a residual ancestral function, and that *in vivo* processing could be mediated by a distinct, as yet unidentified component. There are good grounds for discounting this possibility and arguing that subunit I/PEP is indeed a bifunctional protein. First, isolated subunit I has a very high processing stimulation activity. As judged from the staining of bands on SDS gels, equimolar amounts of subunit I stimulate MPP to maximal activity. Second, subunit I/PEP of *N. crassa* shows considerable similarity to PEP of yeast (Table 1) and is more closely related to yeast PEP than to yeast subunit I. This close relationship is further indicated by the ability of an antibody against yeast PEP to recognize *N. crassa* subunit I/PEP. Third, the two functions of the *N. crassa* subunit I/PEP in respiration and precursor processing may be located on different domains of the protein. The possibility that a putative hydrophilic N-terminal domain points into the matrix and bears the PEP-function is supported by its remarkable similarity to

FIG. 1 Stimulation of the activity of the MPP by PEP, subunits I and II and whole cytochrome reductase.

METHODS. Increasing amounts of PEP, subunits I or II or cytochrome reductase (amount of added protein based on subunit I content) were added to the processing assay (15 μ l), which contained 30 mM Tris-HCl, pH 8.2, 2 mM MnCl₂, 1 mM PMSF and precursor to ATPase F₁F₀ synthesized from the cDNA in pGEM plasmid as substrate⁸. The reaction was started with 50 ng MPP and carried out for 30 min at 25°C. The processing product was analysed by SDS-PAGE, fluorography and quantification by laser densitometry. For isolation of subunit I, 30 mg of a subcomplex of the subunits I and II (ref. 5) was dialysed for 6 h against 50 mM Tris-HCl, pH 8.0, and 50 mM NaCl, and loaded onto a 1.5 $\times$ 35-cm DEAE Sepharose CL-6B column (Pharmacia) in the same buffer. Subunit II passed through; subunit I and undissociated subcomplex were eluted at 200 mM NaCl using a 500 ml gradient from 50–500 mM. Peak fractions were pooled, concentrated 10-fold by ultrafiltration and gel-filtered on a 1.0 $\times$ 50-cm Ultrogel AcA 34 column (LKB) in 50 mM Tris-HCl, pH 7.0. Subunit I (3 mg ml⁻¹) eluted as a protein of relative molecular mass 50,000. PEP was isolated from a mitochondrial extract, prepared by sonication and centrifugation (40,000 r.p.m. for 60 min). The supernatant (50 mg protein) was loaded onto a 2.5 $\times$ 5.0-cm hydroxyapatite column (Biorad) in 10 mM Mops, pH 7.2, 50 mM NaCl and 0.1 mM EDTA. In the presence of a 300 ml gradient from 0–200 mM Na-phosphate, PEP eluted at 110–140 mM. The protein was loaded onto a MonoQ column (Pharmacia) and chromatographed with 10 ml of a gradient from 50–400 mM NaCl. PEP (0.05 mg) eluted at 210–250 mM NaCl and was gel-filtered on a Superose 12 column (Pharmacia) in 50 mM Tris-HCl, pH 7.5, and 100 mM NaCl. MPP was enriched from a cell extract of *N. crassa* hyphae by DEAE cellulose and Zn-Chelat chromatography⁹.

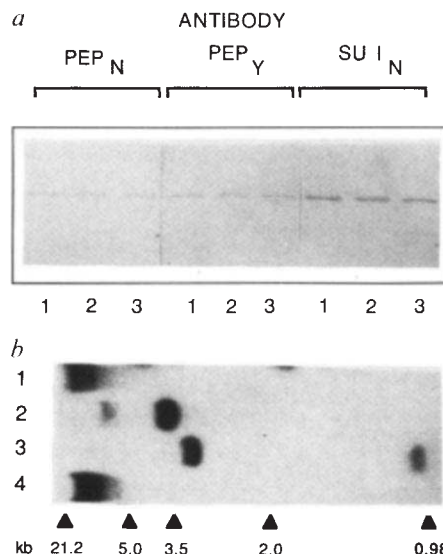
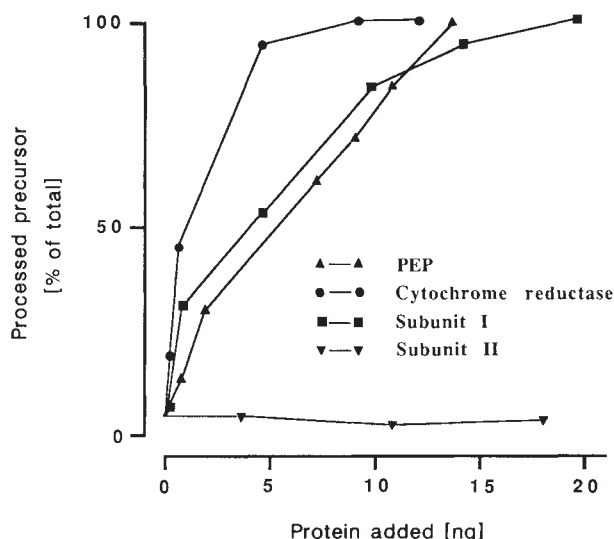


FIG. 2 a, Immunoblotting of PEP (1), subunit I (2) and cytochrome reductase (3) from *N. crassa* with antisera against *N. crassa* PEP (PEP_N), yeast PEP (PEP_Y, *mas1* protein) and *N. crassa* subunit I (SU I_N). b, Southern hybridization of restricted *N. crassa* genomic DNA with PEP cDNA.

METHODS. a, PEP, subunit I, (each 0.1 μ g) and cytochrome reductase (0.5 μ g) were subjected to SDS-PAGE, transferred to nitrocellulose paper¹⁸ and immunodecorated with the different antibodies. Bound antibodies were visualized using alkaline phosphatase coupled to antibodies against rabbit IgG¹⁹. b, 10 μ g of total DNA extracted from *N. crassa* wild-type 74A were restricted with *Bam*HI (lane 1), *Eco*RI (lane 2), *Hind*III (lane 3), *Pst*I (lane 4) and electrophoresed on a 0.8% agarose gel. Fragments were blotted onto nylon filters and hybridized with a nick-translated probe of the PEP cDNA⁹ which represented the complete coding and 3'-untranslated region²⁰. Hybridization was in 10 $\times$ Denhardt's solution, 2 $\times$ SSC, 0.1% SDS at 65°C for 20 h. Washing was performed twice with 0.1% SSC at 65°C for 10 min.

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Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation

Aleksandra Wodnar-Filipowicz, Christoph H. Heusser* & Christoph Moroni

Institute for Medical Microbiology, University of Basel, Petersplatz 10, 4003 Basel, Switzerland

* CIBA-GEIGY Ltd, Pharmaceuticals Research Department, Section Immunology, 4002 Basel, Switzerland

MAST cells have a central role in allergic diseases mediated by specific immunoglobulin E antibody responses to allergens. The binding of IgE to the high-affinity receptor for IgE (FcεR) on mast cells and basophils enables these cells to react specifically to allergens. Such contact leads to the activation of mast cells and the release of histamine and other pharmacological mediators, causing an immediate hypersensitivity and acute inflammatory reactions, accompanied by the development of allergic symptoms^{1,2}. Here we show that FcεR-mediated activation of murine mast cells results in the production of the haemopoietic growth factors granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3). IL-3 and GM-CSF, in addition to their role in bone marrow haemopoiesis, also influence inflammation as they have the capacity to recruit, prime and activate inflammatory cells such as neutrophils, macrophages and eosinophils^{3,4}. Secretion of these factors by mast cells in response to allergens may therefore have an important role in local tissue defense.

Mast cells derived from murine bone marrow cultures (see legend, Fig. 1) were sensitized by coating with monoclonal IgE antibodies against phosphorylcholine (PC) and subsequently activated by exposing cells to the polyvalent antigen PC-conjugated bovine serum albumin (PC-BSA) (ref. 5). Mast-cell activation leading to degranulation was monitored by measuring histamine release (see legend, Fig. 1). Such activation also resulted in the expression of messenger RNAs encoding GM-CSF and IL-3, as determined by northern blot analysis (Fig. 1). Expression of these mRNAs was not detectable in cells from uninduced cultures, or in cells from cultures exposed to antibody or antigen alone (Fig. 1a and b, lanes 1–3). A rapid response was seen, however, when coating with IgE was followed by PC-BSA treatment, as the GM-CSF and IL-3 mRNAs were readily detectable after 30 min (Fig. 1a and b, lane 4). GM-CSF mRNA was present in the form of two distinct bands of about 1.2 kilobases (kb) (Fig. 1a), a pattern also observed in other studies^{6,7}. Induction and accumulation of both GM-CSF and IL-3 mRNAs was transient, as only residual amounts of mRNAs were visible after two hours of induction (Fig. 1a and b, lane 5). Rapid disappearance of these mRNAs is consistent with the low stability reported for GM-CSF mRNA⁸ and postulated for mRNAs encoding other lymphokines^{8,9}. The induced levels of IL-3 mRNA were significantly lower than those of GM-CSF mRNA (20 µg total RNA and 10 µg poly(A)⁺ RNA were analysed per lane in panels a and b, respectively).

We have also determined whether cultures of activated mast cells secrete biologically active GM-CSF and IL-3 (Fig. 2). Conditioned media from cells after stimulation were tested for their ability to support proliferation of IL-3-dependent PB-3c cells¹⁰ (Fig. 2a) and FDC-P1 cells, that are GM-CSF or IL-3 dependent¹¹ (Fig. 2b). Both factors were secreted (Fig. 2a and b, open bars) in a process reflecting the FcεR involvement, as only supernatants from cultures subjected to activation by IgE and PC-BSA (supernatants 4–8) but not those from the control cultures (supernatants 1–3) showed substantial activity. The

kinetics of accumulation of IL-3 and GM-CSF in conditioned media of activated mast-cell cultures differed significantly. IL-3, as measured by growth of PB-3c cells, reached its highest level 30 min after addition of PC-BSA (Fig. 2a, open bars). Thereafter it rapidly decreased, probably reflecting the fact that the IL-3 dependent bone marrow-derived mast cells themselves use their own IL-3. Maximum levels of secreted GM-CSF, as judged by efficient stimulation of growth of FDC-P1 cells, reached their peak 2 h after exposure to PC-BSA and remained unchanged thereafter (Fig. 2b, open bars). This accumulation of GM-CSF in activated bone marrow cultures reflects the fact that GM-CSF is not used as a growth factor by mast cells.

The biological activities of IL-3 and GM-CSF in conditioned media were effectively blocked by anti-IL-3 and anti-GM-CSF antibodies, respectively. The growth of PB-3c cells, dependent

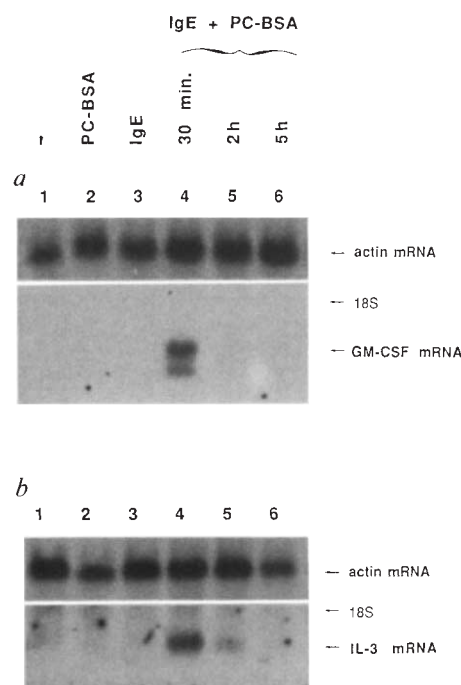


FIG. 1. Effect of Fcε receptor-mediated activation of mast cells on expression of *a*, GM-CSF mRNA and *b*, IL-3 mRNA. Lane 1, untreated cells; lane 2, cells exposed to PC-BSA for 30 min; lane 3, cells exposed to IgE for 30 min; lanes 4–6, cells treated with IgE for 30 min, followed by incubation with PC-BSA for 30 min (lane 4), 2 h (lane 5) or 5 h (lane 6).

METHODS. Bone marrow cells from DBA/2 mice were cultured in Iscove modified Dulbecco medium containing 10% fetal calf serum, supplemented with conditioned medium from the IL-3 producing WEHI-3B cell line. After 3 weeks in culture³⁰, over 95% of the cells were mast cells, as assessed by their morphological appearance after May-Grünwald-Giemsa staining, and the presence of cell surface high-affinity IgE receptors (demonstrated by cytofluorography). Determination of possible contaminating T cells was performed by flow cytometric analysis and revealed no detectable Thy-1 positive cells. The activation procedure was as follows. Cells ($5 \times 10^6 \text{ ml}^{-1}$) were coated with monoclonal IgE antibodies for 30 min at 37 °C, using $20 \mu\text{g ml}^{-1}$ of IgE anti-PC (clone 12-3), cells were then washed three times in IL-3-free medium, resuspended at $2.5 \times 10^6 \text{ cells ml}^{-1}$ and exposed to PC-antigen at 37 °C using $10 \mu\text{g}$ PC-conjugated BSA per ml (ref. 5) for the times indicated. Samples of total cellular RNA ($20 \mu\text{g}$ per lane) (*a*), and poly(A)⁺-selected RNA ($10 \mu\text{g}$ per lane) (*b*) were size-separated and blotted onto nitrocellulose. SP6 transcripts of pSP65-multi-CSF or pSP65-GM plasmids containing cDNA fragments of 368 and 398 base pairs (bp) respectively (kindly provided by N. M. Gough³¹) were used as hybridization probes. All lanes showed comparable hybridization signals when blots were reprobed with the 567-bp actin cDNA fragment (provided by Y. Nagamine). Supernatants of cell samples were analysed for their content of histamine³². Histamine release induced by IgE or PC-BSA alone was less than 10%, whereas treatment with IgE followed by incubation with PC-BSA for 30 min, 2 h or 5 h resulted in a specific histamine release of 30, 67 or 82%, respectively (total histamine content was $53 \text{ ng per } 10^6 \text{ cells}$).

on IL-3, was efficiently inhibited by anti-IL-3, but not by anti-GM-CSF antibodies (Fig. 2a, hatched and solid bars respectively) whereas anti-GM-CSF antibodies inhibited the growth of FDC-P1 cells (Fig. 2b, solid bars). The inhibition by anti-GM-CSF antibodies was seen most prominently in the case of supernatants from cultures activated with IgE and PC-BSA for a relatively long time (5–20 h, supernatants 6–8). The effect of anti-GM-CSF antibodies was less pronounced with media from cultures activated for 30 min or two hours (supernatants 4, 5) because of the considerable amounts of IL-3 present in those media (Fig. 2a).

There is evidence^{1,12} that the high-affinity Fc receptors for IgE on mast cells are coupled to the phospholipase C effector system that controls two distinct signal transduction pathways,

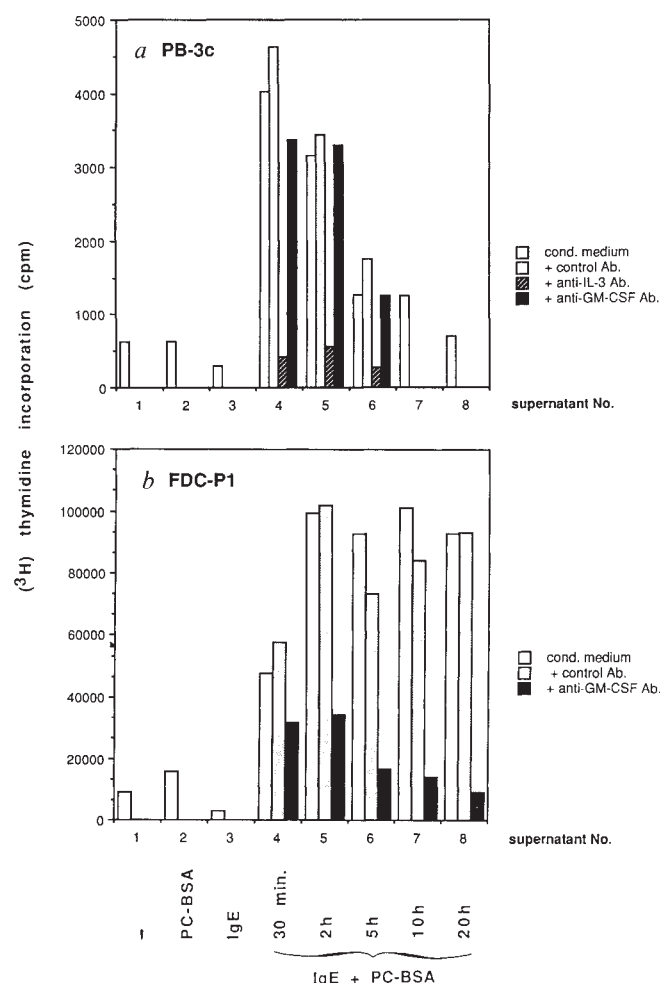


FIG. 2. GM-CSF and IL-3 activities in supernatants from murine bone marrow-derived mast cells activated via Fcε receptors. Bone marrow mast cells, grown in IL-3-containing medium (see legend to Fig. 1), were thoroughly washed before the start of an experiment. IgE coating and PC-BSA treatment (as described in legend to Fig. 1) were done in IL-3 free medium. This treatment allowed the activity of newly produced growth factors in the culture supernatants to be monitored. Supernatants derived from control bone marrow cells (supernatants 1–3) and cells activated for various length of time, as indicated (supernatants 4–8), were cultured with PB-3c (a) and FDC-P1 (b) indicator cells in microtitre plates using 2×10^4 cells per well. After 24 h, cells were pulsed with $0.5 \mu\text{Ci}$ [^3H]thymidine (87 Ci mmol^{-1} ; Amersham) for 6 h before harvesting. For the experiment on antibody inhibition, control rabbit anti-mouse antibodies, anti-IL-3 or anti-GM-CSF antibodies (kindly provided by Dr V. Kindler) were preincubated with the tested supernatants for 2 h at 4°C before addition of FDC-P1 or PB-3c cells. All values are means of triplicate analyses; standard deviations were below 10%.

one regulated by Ca^{2+} ions and the other by protein kinase C (ref. 13). Mast cell functions, such as exocytotic degranulation, are associated with an increased cytoplasmic level of calcium ions^{1,14–16} and, activation of mast cells can be achieved by the use of calcium ionophores¹⁷, which raise Ca^{2+} concentration through a receptor-independent mechanism.

The effect of calcium ionophore A23187 on the expression of GM-CSF and IL-3 mRNAs was compared with the effect of the phorbol ester phorbol-12-myristate-13-acetate (PMA), a reagent activating protein kinase C (Fig. 3). Treatment of bone marrow-derived mast cells with PMA induced some GM-CSF mRNA, as observed before in T lymphocytes⁸, but did not induce IL-3 mRNA (Fig. 3a and b, lane 3). On the other hand, high levels of both mRNAs were expressed in cells treated with calcium ionophore (Fig. 3a and b, lane 2). These results indicate that the Ca^{2+} -dependent signal transduction pathway could be important in regulating the expression of GM-CSF and IL-3 in mast cells. The simultaneous expression of both factors adds to the evidence for the co-ordinate regulation of their genes, which are closely linked on the same chromosome^{18,19}.

The biological activities of GM-CSF and IL-3 show a considerable overlap. Both factors play an essential part in marrow haemopoiesis, supporting stem cells and promoting growth and development of progenitor myeloid lineages of granulocytes, macrophages, eosinophils and megakaryocytes. IL-3 also specifically stimulates the proliferation of mast cells^{4,20} and GM-CSF enhances the growth and functional activity of mature cells of myeloid lineages^{21–24}. GM-CSF and IL-3 are among several lymphokines known to be produced by activated T lymphocytes^{3,20}. GM-CSF is also secreted by macrophages, fibroblasts and endothelial cells in response to various stimuli^{6,25}. On the other hand, production of IL-3 has been previously demonstrated, outside T-lymphocytes, only in transformed cells such as the myelomonocytic leukemia WEHI-3B (ref. 26) and v-Ha-ras-induced autocrine mastocytomas²⁷. Our data strongly suggest that physiologically activated mast cells are a source of both IL-3 and GM-CSF. Without performing *in situ* hybridizations we cannot formally exclude the possibility that contaminating cells were responsible for the synthesis of these lymphokines in our cultures. But we consider this to be most unlikely as our mast cell preparations were $>95\%$ pure. T cells were not detectable (see legend to Fig. 1), and the expression of IL-3 and GM-CSF was dependent on mast-cell activation.

Secretion of these factors by activated mast cells may be important for tissue responses following contact with an allergen and for the body defence against parasitic infections. GM-CSF,

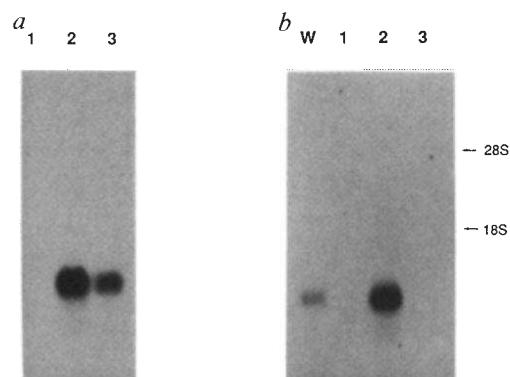


FIG. 3. Comparison of the effects of the calcium ionophore A23187 and PMA on the expression of GM-CSF (a) and IL-3 (b) mRNAs in mast cells. Lane 1, untreated cells; lane 2, cells treated for 3 h with $5 \mu\text{M}$ A23187; lane 3, cells treated for 3 h with 20 ng ml^{-1} PMA; lane W, IL-3 mRNA-expressing WEHI-3B cells. Total cellular RNA ($15 \mu\text{g}$ per lane) was analysed by the northern blot technique using the probes described in the legend to Fig. 1.

synthesized locally at inflammatory sites by mast cells, would attract, maintain growth and stimulate granulocytes, macrophages and eosinophils, thus ensuring the retention and activation of these cells at the region of inflammation. Secreted IL-3 would support continuous proliferation of mast cells. It has recently been shown that fetal liver-derived non-activated mast cells express mRNA for interleukin-4 (BSF-1), a B cell growth factor²⁸, known to promote the isotype switching of B cells to IgE (ref. 29). Taken together with our data on IgE-dependent production of IL-3, these results imply that mast cells actively modulate, both indirectly and directly, their own growth and functional activity.

In summary, as a result of secretion of growth factors, mast cells may play a far more important part in allergic and immune responses than has been assumed so far. Secretion of lymphokines accompanying FcεR-mediated activation of mast cells would enable the local defence system to become self-regulated. This may have major implications in diseases such as allergic asthma. Whether mature human blood basophils and/or tissue mast cells also have the capacity to generate these lymphokines in response to allergen stimulation is currently under investigation. □

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Suppressor effects and cyclic AMP accumulation by the CD29 molecule of CD4⁺ lymphocytes

Hervé Groux, Stéphane Huet, Hélène Valentin,
Danièle Pham & Alain Bernard

Laboratoire d'Immunologie des Tumeurs de L'Enfant,
Institut Gustave-Roussy, 94805 Villejuif Cedex, France

INTEGRINS are a superfamily of related molecules whose function, where known, is to mediate adhesion. The so-called very-late-activation antigen (VLA) family includes at least five distinct heterodimers, each composed of a unique α -subunit non-covalently associated with a common β -subunit. Several members of the family have been shown to bind extracellular matrix proteins, but the function of VLA-4 is so far unknown^{1–4}. VLA-4 is the only member of the family detected on thymocytes and resting T cells. We show here that an antibody which recognizes the β -subunit of VLA-4 (CD29) on T cells can inhibit CD4⁺ cell proliferation triggered by CD2 or CD3, and that binding of this antibody to activated T cells leads to an increase in cyclic AMP levels which is comparable to that elicited by forskolin. These negative signalling effects are unique to this antibody: other CD29 antibodies do not affect the growth of activated CD4 cells but enhance the proliferation of whole T cell populations and abrogate the suppressive effects of mitomycin-treated CD8 cells on CD4-cell growth. Taken together, our results indicate that VLA-4 functions in cell–cell interactions and that it is the target for the suppressive effects of CD8 cells on CD4 cells.

We used eight anti-VLA monoclonal antibodies, all of which have a similar pattern of tissue distribution and react with thymocytes. Some are already well defined: K20 and 4B4—both of which were initially used to define CD29 (ref. 5)—and TS2/16 all recognize the common β -subunit⁶, whereas HP2/1 recognizes the α^4 -subunit⁶. We also used another antibody, O403, which immunoprecipitates the same molecular complex as HP2/1 (Fig. 1)³ and the same molecule as K20, as demonstrated by sequential

TABLE 1 Effect of CD29 antibody on cAMP in T cells and CD4⁺ cells*

		cAMP at 10 min (fmol per 3 × 10 ⁵ cells)	cAMP at 30 min (fmol per 3 × 10 ⁵ cells)
Activators			
Effectors			
T cells			
None	None	350 ± 130	380 ± 160
Idem	K20 (50 µg ml ⁻¹)	345 ± 105	335 ± 140
Idem	O403 (50 µg ml ⁻¹)	330 ± 120	365 ± 130
CD3 antibody crosslinked + rIL-1 (100 U ml ⁻¹)			
Idem	None	620 ± 140	410 ± 120
Idem	K20 (50 µg ml ⁻¹)	945 ± 85	1,510 ± 230
Idem	O403 (50 µg ml ⁻¹)	560 ± 220	410 ± 80
GT2 + T11 ₁ + rIL-1 (50 U ml ⁻¹)			
Idem	None	520 ± 130	410 ± 80
Idem	K20 (50 µg ml ⁻¹)	1,105 ± 130	1,420 ± 160
Idem	O403 (50 µg ml ⁻¹)	495 ± 140	350 ± 130
None	Forskoline (5 × 10 ⁻⁴ M)	1,120 ± 130	1,630 ± 150
CD4 ⁺ cells			
None	None	325 ± 140	410 ± 220
Idem	K20 (50 µg ml ⁻¹)	330 ± 170	395 ± 145
Idem	O403 (50 µg ml ⁻¹)	380 ± 160	420 ± 150
CD3 antibody crosslinked + rIL-1 (100 U ml ⁻¹)			
Idem	None	540 ± 120	430 ± 150
Idem	K20 (50 µg ml ⁻¹)	890 ± 130	1,440 ± 230
Idem	O403 (50 µg ml ⁻¹)	490 ± 150	380 ± 140
None	Forskoline (5 × 10 ⁻⁴ M)	980 ± 180	1,560 ± 210

* cAMP was assayed by radioimmunoassay. Briefly, cells were washed three times with RPMI 1640 and resuspended in the same buffer at a concentration of 1.5 × 10⁶ cells per ml in the presence of different effectors. After incubation at 37 °C, the reaction was stopped by heating at 80 °C for 3 min. Samples were then treated with 1 ml ethanol, centrifuged and the supernatants collected. After evaporation, the residues were dissolved in PBS and cAMP was assayed using a cAMP-[¹²⁵I] assay system (dual range) (Amersham). Results are expressed in femtomoles ± s.d. (n = 4).

immunoprecipitation from lysates of ¹²⁵I-surface-labelled thymocytes (Fig. 1). Thus all our antibodies react with the VLA-4 molecule on resting T cells. From competitive binding experiments, we have shown that K20 reacts with a distinct epitope compared with the other monoclonal antibodies used here (data not shown).

K20, or its F(ab')₂ fragment, inhibited T-cell proliferation induced by a monoclonal antibody against CD3, by two different pairs of CD2 antibody, or by TPA(12-*O*-tetradecanoylphorbol-13-acetate) plus ionomycin (Fig. 2a and c). Thus it counteracts the effects of many stimulating agents, including those active at the post-receptor level. Moreover, the inhibitory effect of K20 is directly exerted on T cells, as it was observed when highly purified peripheral blood T lymphocytes were stimulated in the presence of recombinant interleukin 1 (rIL-1). K20 inhibited synthesis of interleukin 2 (IL-2) (Fig. 3); it also blocked the appearance of IL-2 receptors (IL-2-R), but only when T cells were stimulated by CD3 and not by CD2 (Fig. 3). This is in agreement with our data on [³H]thymidine incorporation in the presence of K20: strong inhibition when T cells are activated by CD3 and partial inhibition on activation by CD2 (Fig. 2c). It is also consistent with our previous observations regarding T-cell activation by CD2 as compared with CD3, namely that distinct primary signals of activation are delivered to the T cell by these molecules^{7,8}. When we tested K20 on sorted CD4⁺ cells, we found that it still blocked their proliferation (Fig. 2d).

The inhibitory signal transmitted by the CD29 molecule is most probably mediated by cAMP, because we found that K20 induces a rapid, high and durable increase of cytosolic cAMP,

maintained for up to 60 min (Table 1). The level of cAMP attained is comparable to the level measured in the positive control in which cells were incubated with forskolin, a direct and potent activator of adenylate cyclase. For this rise to occur, T or CD4⁺ cells need, in addition to K20, to be activated either by a CD3 antibody or by a pair of CD2 antibodies. Activation of adenylate cyclase, either directly with forskolin⁹ or with prostaglandin PGE₂ (ref. 6), enhances cAMP turnover, leading to diminution of IL-2 synthesis, inhibition of lectin-stimulated polyphosphoinositide turnover¹⁰ and inhibition of T-cell proliferation. Indeed K20, like PGE₂, inhibits IL-2 synthesis in Jurkat cells by increasing the cytosolic cAMP level (data not shown). The increase of cytosolic cAMP induces suppressive effects on the immune response (for review, see ref. 11).

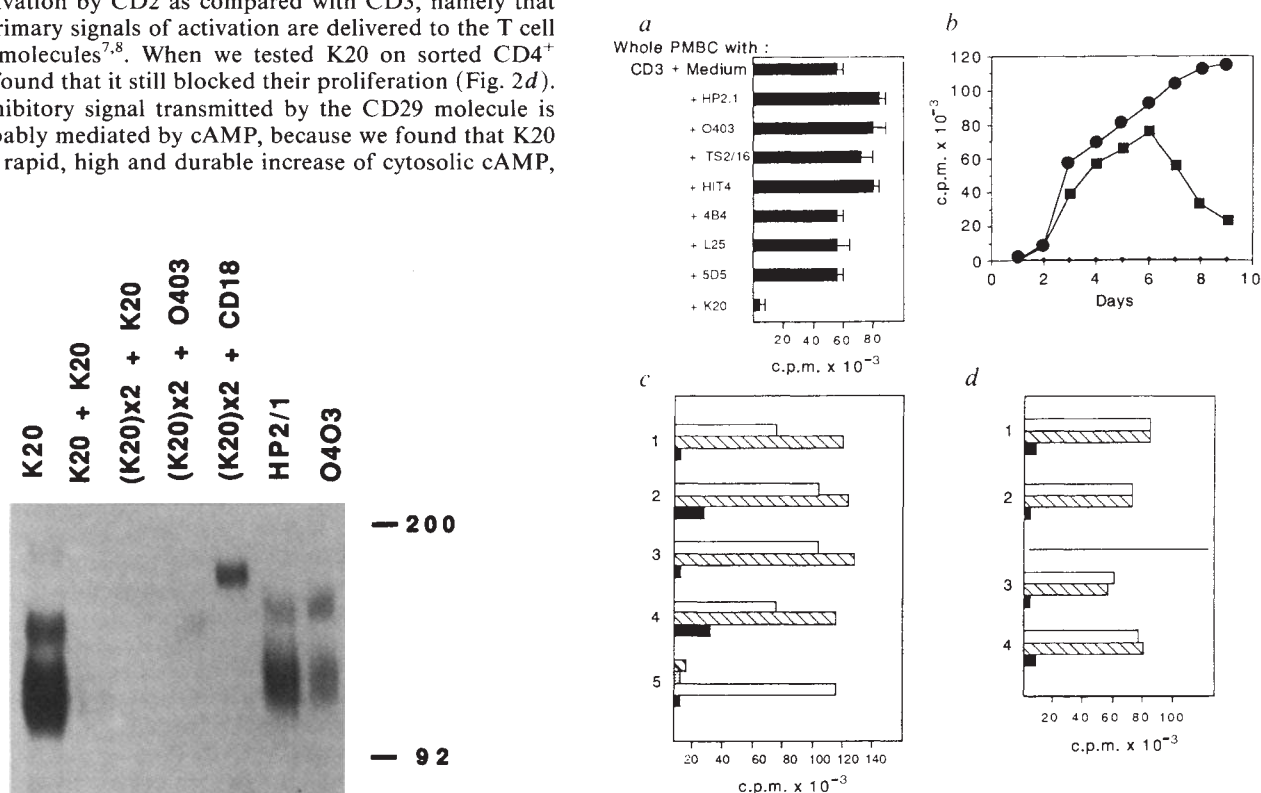
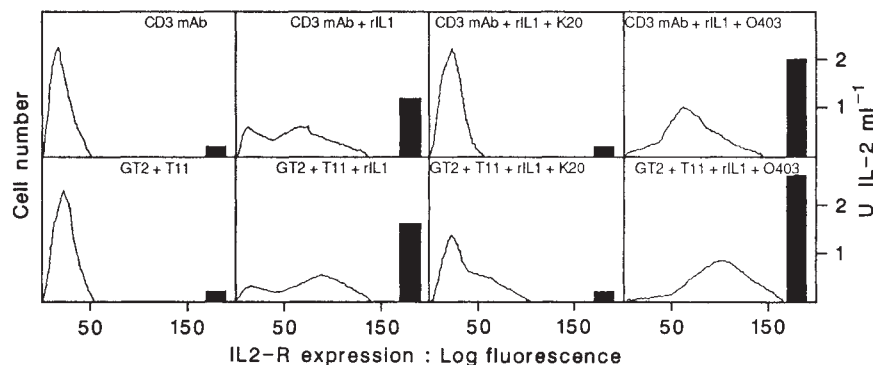


FIG. 2 Effect of a battery of CD29 antibodies on T-cell activation. Eight CD29 antibodies were tested. **a**, [³H]thymidine incorporation, measured at day 3, by whole peripheral blood mononuclear cells (PBMC) stimulated with a soluble CD3 (X35.7) in the presence of 10 μg ml⁻¹ of CD29 antibody or in medium alone. **b**, Kinetics of [³H]thymidine incorporation by highly purified T cells⁷ stimulated with CD3 antibody coated on culture plates²⁰ plus rIL-1 (100 U ml⁻¹), in the presence of CD29 O403 (●) (5 μg ml⁻¹), CD29 K20 (+) (5 μg ml⁻¹; all points fall on abscissa), or an irrelevant antibody (CD1 D47) (■). **c**, Effect of O403 (5 μg ml⁻¹) (cross-hatched; 1-4) and K20 used as F(ab')₂ fragments (filled bars; 1-5)²¹ (10 μg ml⁻¹) on whole PBMC (1 and 2) or on highly purified T cells (3-5). Whole PBMC were stimulated with either soluble CD3(1) or two typical mitogenic pairs of CD2 antibodies (2) (ref. 7). Purified T cells were stimulated with either CD3 coated on culture plates²⁰ plus rIL-1 (Glaxo); 100 U ml⁻¹ (3) or with two particular mitogenic pairs of CD2 (ref. 7) plus rIL-1 (50 U ml⁻¹) (4) or with TPA (10⁻¹¹ M) plus ionomycin (60 μg ml⁻¹) (5) at a concentration such that no proliferation could occur with these agents alone (thick cross-hatched and dotted bars respectively). [³H]Thymidine incorporation was measured on day 3, when T cells were stimulated by CD3, and on day 4 when T cells were stimulated by CD2. **d**, Effect of CD29 antibodies on proliferation of sorted T-cell subsets (top panel, CD4⁺ cells; lower panel, Leu 15⁻ cells). CD4⁺ cells were selected for negatively using CD8 X8 antibody plus rabbit complement²². Leu 15⁻ cells were isolated by cell-sorter analysis (Leu 15 purchased from Becton Dickinson). The different subsets were stimulated as T cells and CD29 antibodies were used and designated as in c.

FIG. 1 Western blot to characterize the molecule recognized by O403 on T cells. K20 (γ2a) and O403 (γ1) were isolated as described¹⁸; HP2/1 was donated by F. Sanchez-Madrid³, and reacted with the VLA-4 heavy chain (ref. 1). Monoclonal antibody K20 was ascribed to CD29 by the agreement in ref. 5. The results of immunoprecipitation and sequential immunoprecipitation on lysates from ¹²⁵I-surface-labelled thymocytes⁴ are shown using K20 (lane 1), HP2/1 (lane 6), O403 (lane 7), and with K20 after one or two cycles of pre-clearing with K20 (lanes 2 and 3 respectively), with O403 after two cycles of pre-clearing with K20 (lane 4), or with CD18 M232 (ref. 9) after two cycles of pre-clearing with K20 for comparison (lane 5). Molecular weight calibration (K) is shown on the right.

FIG. 3 Effect of CD29 on IL-2-R expression and IL-2 synthesis detected in T cells stimulated via CD3 or CD2. Purified T cells were stimulated for 3 days with the CD3 antibody X35.7, previously coated on culture plates or with the CD2 GT2 + T11₁ pair⁷, both in the presence of rIL-1 (100 U ml⁻¹). Monoclonal antibodies were added at 5 µg ml⁻¹ at the start of culture. IL-2-R expression was revealed with a directly fluoresceinated CD25 (IOT14) antibody (Immunotech, Marseilles). Log fluorescence was analysed by cytofluorometry (Ortho System 50H). Supernatants of stimulated T cells were tested after 24 h for IL-2 activity by measuring the proliferation of the IL-2-dependent CTL-L2 cell line²³.



At this stage, we assumed that K20 exerts an agonistic effect on CD29, which might act as a receptor for a suppressive effect on T-cell proliferation. We therefore searched for other anti-VLA antibodies that would exert effects opposite to those of K20. We found that 4 out of the 7 other antibodies tested enhanced [³H]thymidine incorporation by whole peripheral blood mononuclear cells or by highly purified T cells that had been stimulated via either CD3 or CD2 (Fig. 2a and c). The 'enhancing' monoclonal antibodies, which include HP2/1 reacting with the α_4 -chain, act by increasing the amount of IL-2 released and the number of cells carrying the IL-2-R (Fig. 3).

We also observed a profound change in the kinetics of T-cell proliferation stimulated by mitogenic CD3 antibodies plus rIL-1: an enhancing mAb, O403, induces a continuous increase in the level of [³H]thymidine incorporation over 9 days of culture (Fig. 2b). It should be noted that this dramatic change in the kinetics of [³H]thymidine incorporation could only be seen under these particular conditions. The enhancing anti-VLA antibodies could exert their effect only when the whole T-cell population is cultivated, and not just on separated CD4⁺ or Leu 15⁻ subsets (Leu 15 is a T-suppressor cell marker¹²), that is, in the absence of suppressor T cells (Fig. 2d). In addition, the enhancing antibody abrogates the suppression of the proliferation of CD4⁺ cells exerted by mitomycin-treated CD8⁺ cells (Table 2). This action must be directly targeted at CD4⁺ cells as the inhibition is still effective with a large excess of mitomycin-treated CD8⁺ cells.

Thus it seems that the CD29 molecule acts as a receptor

controlling CD4⁺-cell proliferation. K20 would mimic the fixation of the 'physiological' ligand of CD29 that issues from CD8⁺ cells, which could be either a surface molecule or a soluble ligand, whereas the enhancing antibodies block its function. This conclusion explains observations on the blocking effect of the CD29 antibody 4B4 on the suppression induced by CD8⁺ cells on the specific immunoglobulin E response induced by CD4⁺ cells¹³, as well as our data reported here.

Within the CD4⁺ T-cell population, 4B4, together with the CD45R antibody 2H4, has enabled two reciprocal subsets to be distinguished: the CD4⁺ CD29⁺ helper-inducer subset; and the CD4⁺ CD45R subset acting as suppressor-inducer^{14,15}. In view of recent data showing that the CD29⁺ subset (CD29^{high}) and the reciprocal CD45R⁺ subset (CD45R^{high}) represent memory and naïve cells^{16,17} respectively, one can speculate on the role of the CD29 molecule in the regulation of the immune response. As naïve CD4⁺ cells tend to inhibit, and memory CD4⁺ cells vigorously induce, antibody production, the increase in the density of CD29 molecules could act to create a condition that renders them susceptible to control by CD8⁺ cells. This fits with our observation that CD4⁺ cells must be activated to induce the inhibitory rise of cAMP. Because one of the primary functions of the CD29 molecule on CD4⁺ T cells would be to mediate an increase in levels of cAMP, we can see why functionally specialized leucocyte subsets other than CD4⁺ cells would carry this molecule. K20, or eventually the natural ligand that it mimics, might be a candidate to artificially induce immunosuppression. □

TABLE 2 Effect of CD29 antibody on [³H]thymidine incorporation by untreated CD4⁺ cells co-cultured with mitomycin-treated cells. TS2/16 is also a CD29 antibody

Cells co-cultured with CD4 ⁺ cells (4 × 10 ⁴)		Medium	0403	CD29 antibody HP2/1	TS2/16
Mitomycin-treated CD8 ⁺ cells					
000	52,217	59,875	52,164	58,671	
2 × 10 ⁴	50,817	48,916	58,714	42,157	
4 × 10 ⁴	40,916	49,875	51,795	39,854	
8 × 10 ⁴	32,824	51,897	56,472	54,153	
12 × 10 ⁴	12,715	48,796	49,257	50,479	
16 × 10 ⁴	8,712	45,795	42,158	45,879	
Mitomycin-treated CD4 ⁺ cells					
16 × 10 ⁴	51,876	48,876	53,289	57,894	

The sorted CD4⁺ cells were stimulated with a coated CD3 antibody plus rIL-1 (50 U ml⁻¹). Increasing amounts of mitomycin-treated CD8⁺ or CD4⁺ cells were added at the start of the culture, either alone or in the presence of different CD29 antibodies (5 µg ml⁻¹). The results are given in c.p.m. and represent the means of triplicates of one experiment representative of 3. We excluded the possibility that suppression might arise from IL-2 consumption, because mitomycin-treated CD4⁺ cells did not show an inhibitory effect, and mitomycin-treated CD8⁺ did not suppress the IL-2 response of the cytotoxic T lymphocyte-L2 line (not shown).

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Tyrosine kinase receptor indistinguishable from the *c-met* protein

S. Giordano*, C. Ponzetto*, M. F. Di Renzo*,
C. S. Cooper† & P. M. Comoglio*‡

* Department of Biomedical Sciences and Oncology, University of Torino, C.so Massimo D'Azeglio 52, 10126 Torino, Italy

† Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, UK

GROWTH factor receptors with protein tyrosine kinase activity are central to the control of proliferation of both normal and malignant cells¹⁻³. Using anti-phosphotyrosine antibodies⁴, we have previously identified a transmembrane glycoprotein with abnormally high protein tyrosine kinase activity in a human gastric tumour cell line (GTL-16)^{5,6}. Electrophoresis under non-reducing conditions revealed that this kinase (relative molecular mass 145,000 (145 K)) is disulphide-linked to a 50K chain in an $\alpha\beta$ -complex of 190K (p190). From its novel two-chain structure, we deduced that p190 was the prototype of a new class of tyrosine kinase receptors. We now show that p190 is indistinguishable from the protein encoded by the *c-met* proto-oncogene and that the $\alpha\beta$ -subunit structure is conserved in other human cell lines. We also show that the high level of p190 found in the GTL-16 cell line is accompanied by amplification and overexpression of *c-met*. This provides the first example of a functional alteration of *c-met* in a human tumour cell line.

P190 is a heterodimer of two disulphide-linked subunits: a 50K α -chain which is exposed at the cell surface and a 145K β -chain that spans the plasma membrane and includes sites for tyrosine phosphorylation^{5,6}. Recently, it has been proposed that the protein encoded by the *c-met* proto-oncogene may have a heterodimeric structure. The model was largely based on the existence of a consensus sequence for proteolytic cleavage (Lys-Arg-Lys-Lys-Arg-Ser) within the predicted *c-met* primary structure⁷.

‡ To whom correspondence should be addressed.

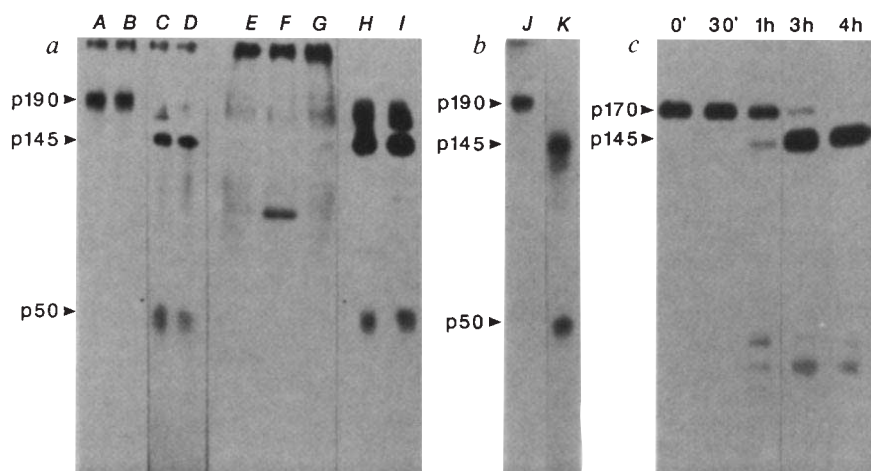
Here we investigate the relationship between p190 and the *c-met*-encoded protein (c-Met) by electrophoresis on SDS-polyacrylamide gels of the proteins immunoprecipitated from GTL-16 cells by anti-phosphotyrosine and anti-c-Met antibodies⁸. When the proteins were extracted and analysed under non-reducing conditions, each antibody precipitated a 190K protein, but under reducing conditions the protein was smaller, running at 145K (Fig. 1, lanes A-D). The 190K proteins identified by each antibody were excised from gels, treated with β -mercaptoethanol and re-examined by SDS-PAGE: they both yielded subunits of 145K and 50K (Fig. 1, lanes H-I). The 145K subunits immunoprecipitated by anti-phosphotyrosine and anti-c-Met antibodies produced identical phosphopeptides after phosphorylation *in vitro* and digestion with V-8 protease or trypsin (Fig. 2). As expected, some of phosphopeptides were labelled with different intensities during the *in vitro* reaction, depending on which antibody was used for immunoprecipitation. Altogether these results demonstrate that the c-Met protein is a heterodimer of α - and β -subunits and is indistinguishable from the p190 that we previously detected in GTL-16 cells. The dimeric structure of the c-Met protein is conserved in other human cell lines such as CALU-1 (Fig. 1, lanes J and K) and A431 (not shown).

Pulse-chase experiments performed on GTL-16 cells using anti-c-Met antibodies showed that the 145K β -subunit originates from a precursor of 170K (Fig. 1c). From sequence data⁹ we infer that the 50K α -subunit might also originate from cleavage and post-translational processing of this precursor.

Growth factor receptors that have a protein tyrosine kinase domain are currently classified into three groups on the basis of common structural motifs^{1,2}. Although the Hanks evolutionary tree places c-Met close to the insulin receptor³, the latter is an $\alpha_2\beta_2$ tetramer. Thus c-Met seems to be the prototype of a fourth class of $\alpha\beta$ dimeric receptors.

Activation of the *c-met* proto-oncogene by rearrangement has been observed in a human osteosarcoma line following treatment with a chemical carcinogen¹⁰⁻¹². GTL-16 cell DNA showed a 10-fold amplification of the non-rearranged c-Met gene (Fig. 3a). Northern analysis of poly(A)⁺ messenger RNA revealed high levels of *c-met* transcripts of sizes 9.0, 7.0, 5.2 and

FIG. 1. Proteins immunoprecipitated from GTL-16 human gastric carcinoma cells and CALU-1 human lung carcinoma cells using anti-phosphotyrosine and anti-c-Met antibodies. **a**, Proteins solubilized from metabolically labelled GTL-16 cells (lanes A-I) were immunoprecipitated by anti-c-Met (lanes A, C, E) or anti-phosphotyrosine (lanes B, D, G) antibodies and analysed by SDS-PAGE, either in the absence (lanes A, B) or in the presence (lanes C-G) of β -mercaptoethanol. Lane E, anti-c-Met antibodies blocked by the competing peptide (1 mM). Lane F, pre-immune rabbit serum. Lane G, anti-phosphotyrosine antibodies absorbed by phosphotyrosine (5 mM). Analysis of p190 after reduction of disulphide bonds (lanes H and I). The p190 bands detected in non-reducing gels of anti-c-Met (as in lane A) and anti-phosphotyrosine immunoprecipitates (as in lane B) were excised. Gel slices were boiled in SDS buffer with β -mercaptoethanol and the eluted proteins separated by SDS-PAGE. Lane H, products of the partial reduction of p190 immunoprecipitated with anti-c-Met antibodies; lane I, products of the partial reduction of p190 immunoprecipitated with anti-phosphotyrosine antibodies. In this type of analysis, reduction is seldom complete and, accordingly, some unreduced p190 is detected. **b**, Cell-surface proteins of CALU-1 cells were labelled with ¹²⁵I by the lactoperoxidase-glucose oxidase method. After solubilization, proteins were immunoprecipitated by anti-c-Met antibodies and analysed in the absence (lane J) or in the presence (lane K) of β -mercaptoethanol. **c**, Pulse-chase experiments performed with GTL-16 cells incubated for 15 min in the presence of [³⁵S]methionine (100 μ Ci ml⁻¹). Numbers indicate the time elapsed after



chasing. Electrophoresis in SDS-PAGE was performed under reducing conditions.

METHODS. Cells were metabolically labelled either overnight with 100 μ Ci ml⁻¹ of [³H]D-glucosamine-HCl (lanes A-G) or for 3 h with 50 μ Ci ml⁻¹ [³⁵S]methionine in methionine-free medium (lanes H, I). Cell-surface iodination, protein extraction, immunoprecipitation and SDS-PAGE analysis were performed as described^{5,13}. Anti-c-Met antibodies were raised against a peptide corresponding to the non-conserved C-terminal tail of the human c-Met protein⁸.

FIG. 2. Phosphopeptide maps of the 145K subunits immunoprecipitated by anti-c-Met antibodies (lane 1 and panel 1a) or by anti-phosphotyrosine antibodies (lane 2 and panel 2a). *a*, One-dimensional phosphopeptide map of the 145K proteins partially digested with V8 protease. *b*, Two-dimensional phosphopeptide map of the same proteins subjected to exhaustive digestion with trypsin. The different amounts of phosphate incorporated in some peptides (such as those numbered 8 and 9) is due to the interference of anti-phosphotyrosine antibodies with the *in vitro* phosphorylation.

METHODS. The 145K subunits were precipitated by anti-c-Met or anti-phosphotyrosine antibodies and labelled *in vitro* by autophosphorylation⁵. Partial proteolytic peptide mapping¹⁴ used 0.5 µg per lane of V8 protease. For two-dimensional maps, labelled proteins were resolved on one-dimensional gels, extracted and subjected to exhaustive tryptic digestion¹⁵. Peptides were separated on cellulose thin-layer plates by electrophoresis at pH 1.9, followed by ascending chromatography in pyridine-acetic acid-butanol-water (4:1.2:6:4.8 v/v). Each cellulose plate was used to compare two digests, which were loaded respectively at 12 and 8 cm along the horizontal axis bisecting the plate. Origins correspond roughly to the positions of phosphopeptides number 4. Electrophoresis along the vertical axis of the plate was at 1,200 volts for 30 min. The wet plate was cut along the central vertical axis and the two halves were subjected to ascending chromatography as indicated in Fig. 2. The pair of tryptic digests therefore generate phosphopeptide maps which are mirror images.

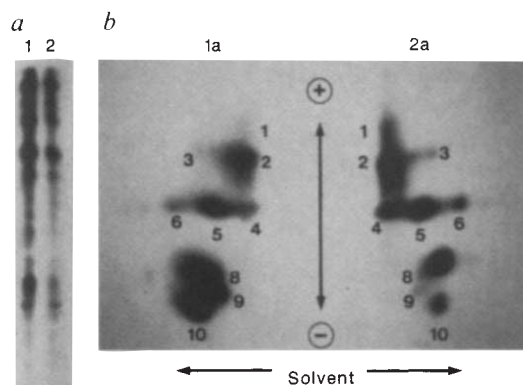
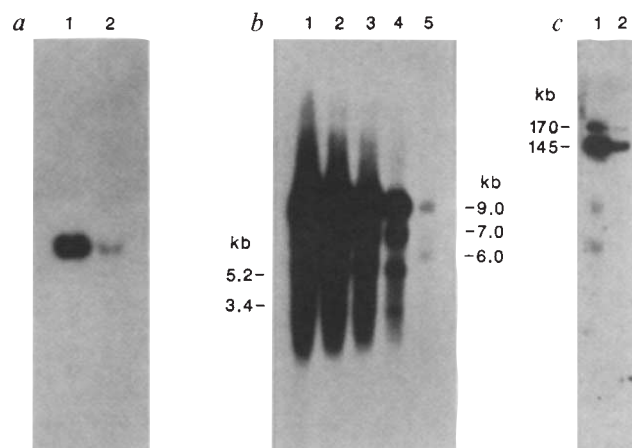


FIG. 3. Amplification and overexpression of *c-met* in GTL-16 cells. *a*, Southern blot analysis of DNA (loading 20 µg per well) extracted from GTL-16 cells (lane 1) and human liver (lane 2) digested with *Eco*RI and probed with *met*-H¹². *b*, Northern blot of poly(A)⁺ RNA obtained from GTL-16 (lanes 1–4) and HOS (lane 5) cells and probed with *met*-G¹². Loadings were 20, 10, 5 and 2.5 µg GTL-16 poly(A)⁺ mRNA in lanes 1, 2, 3 and 4 respectively, and 25 µg HOS poly(A)⁺ mRNA in lane 5. Sizes (kb) were calculated using ribosomal RNAs as standards. *c*, Immunoblot of proteins solubilized from GTL-16 (lane 1) and epidermoid carcinoma A431 (lane 2) cells, probed with anti-c-Met antibodies. Total protein (200 µg per well) was run under reducing conditions.

METHODS. High-molecular weight chromosomal DNA was prepared according to Herrman and Frischauf¹⁶. Restriction enzyme digestion, agarose gel electrophoresis and Southern blotting were performed according to Maniatis *et al.*¹⁷. Southern blots were washed under conditions of high stringency (0.1 × SSC at 68 °C). RNA was prepared according to Cathala *et al.*¹⁸ and formaldehyde agarose gel electrophoresis, northern transfer and hybridization were according to Wahl *et al.*¹⁹. Hybridization was at high stringency (in 50% formamide; the filter was washed in 0.1 × SSC at 65 °C). For immunoblot analysis, cells were solubilized in boiling Laemmli buffer²⁰ with β-mercaptoethanol. Samples were analysed by western blotting as described^{21,22}; blots were probed with anti-Met antibodies and then with ¹²⁵I-labelled *Staphylococcus A* protein A (Amersham).



3.4 kilobases (kb). (Fig. 3b); these probably account for the abundant expression of the c-Met protein (Fig. 3c). The 9.0-kb and the 7.0-kb mRNAs have been detected in a human osteosarcoma cell line (HOS)¹², although the 5.2-kb and the 3.4-kb transcripts are unique to the GTL-16 cell line. These results provide the first example of amplification and overexpression of *c-met* in a human tumour cell line and suggest the possible involvement of this oncogene in gastric tumours. □

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Sleuthing out bacterial identities

B.R. Bochner

Global phenotype analyses are now possible using a versatile redox chemistry that is readily automated with computerization and microplate technology.

CELLS express a very large number of phenotypes, yet tools have not been available to facilitate a comprehensive survey of their specific characteristics — a global phenotype analysis. Such a survey of phenotypic traits requires technology that allows hundreds or thousands of traits to be scanned easily, rapidly and accurately. This capability would open up opportunities for finding the unique traits of individual organisms and for recognizing traits common to groups of organisms, such as species. Global phenotype analysis would also complement recently developed tools for the global analysis of the constituents of a cell, such as chromosomes, nucleic acids, proteins and fatty acids.

The potential applications of global phenotype analysis extend to a wide variety of disciplines and practices. Geneticists constructing cell lines through a defined series of mutations would be able to analyse the phenotypic consequences of genetic alterations. Physiologists seeking to exploit the metabolic potential of microbes for the biosynthesis of small molecules or the degradation of undesirable chemicals could survey many properties of large strain collections. Industrial microbiologists utilizing production strains — such as those used to produce cheese, antibiotics, amino acids or recombinant proteins — could monitor their stock cultures to prevent subtle, deleterious changes. Human, veterinary and plant pathologists could recognize particular pathogenic strains to recommend appropriate therapies and trace epidemics. Global phenotype analysis would also be invaluable for taxonomic, evolutionary and environmental studies. Very little is known about the populations of microorganisms that constitute the human flora, and far less has been gleaned about the number, range and diversity of microbial species on our planet — global phenotypic analysis would provide much-needed insights.

Bacteria and yeast have traditionally been identified by analysing a series of heterogeneous phenotypes, primarily morphologic and metabolic properties. Expansion of the field has been greatly limited because the tools for performing these analyses are cumbersome and inadequate. For example, morphologic descriptions made from observations of single cells or colonies lack sufficient detail to permit species or even genus-level identifications. Metabolic testing has

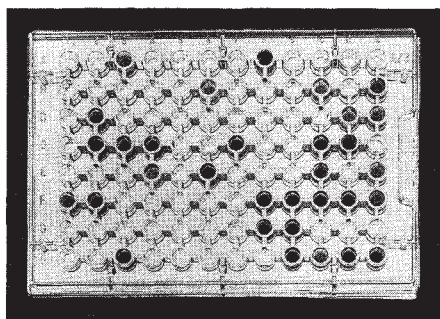


FIG. 1 The metabolic fingerprint pattern generated by *Leminorella grimonii* in Biolog's GN MicroPlate.

adequate resolution, but it is laborious and time-consuming. Biolog, Inc. has developed a new approach which shows great promise in speeding, simplifying and standardizing the process of metabolic testing.

You are what you eat

Biolog has developed a technology that can simply and efficiently characterize and identify microbial isolates by examining their carbon source utilization profiles. The original basis for the approach was first put forward in 1926 in the doctoral thesis of the Dutch microbiologist L. E. den Dooren de Jong¹. Den Dooren de Jong sought to profile and identify an organism according to its catabolic enzymes, which can be examined by looking at what chemicals the organism uses as a carbon and energy source. To be effective, such profiles must include a large and diverse set of chemical substrates. Biolog has made this approach much more tractable by developing a redox-based chemistry which reveals carbon source utilization reactions by a colour change. The simple colour change can be analysed easily by widely available and inexpensive computerized microplate technology.

Metabolic fingerprinting

The scientific foundations of Biolog's redox chemistry are elaborated in a 1977 publication by Bochner and Savageau² and in two US patents^{3,4}. A redox dye such as tetrazolium violet is used to detect colorimetrically the increased metabolic rate of a cell which is oxidizing a carbon source. Thus, if a cell is presented with a chemical that it can oxidize, its metabolism increases and the colourless dye is irreversibly reduced to a purple formazan. If the cell is given a chemical that it cannot oxidize, its metabolic rate does not

increase and no colour change occurs. When different carbon sources are fed to cell suspensions in different wells of a microplate, the result is a pattern of purple and colourless wells in an 8 × 12 matrix — the organism's "metabolic fingerprint" (Fig. 1).

Regardless of its structure, virtually any chemical substrate that is oxidized by a cell will result in the formation of NADH, which donates electrons to the electron transport chain. Redox dyes such as tetrazoliums tap electrons from this flow, converting the tetrazolium to a highly coloured formazan.

Although the structures of the electron transport chains of various microorganisms differ widely, the tetrazolium redox chemistry can be optimized to work for virtually every species of bacteria and yeast. A fundamental aspect of Biolog's redox chemistry is its universality: it functions independently of the specific structure of the electron transport chain. Even anaerobic bacteria have electron transport mechanisms which can be diverted to reduce tetrazolium.

Because Biolog's chemistry detects a response keyed to the cell's metabolic rate, it can be used to test not only for the utilization of traditional carbohydrates, but for the utilization of virtually any biodegradable chemical. The test's general applicability dramatically increases the number of carbon sources that may be used in an identification panel, from the 50 common carbohydrates currently used in pH-based fermentation tests, to well over 10,000 compounds of many diverse classes. The vast array of potential chemical substrates includes carbohydrates, carboxylic acids, amides, esters, amino acids, peptides, amines, alcohols, aromatic chemicals, halogenated chemicals, phosphorus- and sulphur-containing chemicals and polymeric chemicals.

Biolog has developed a directed, systematic process for selecting sets of substrates that can be used to identify and characterize unknown microbes with a high degree of accuracy. Because the technology detects increases in metabolic rates, it should be possible to modify the chemistry to expand the scope of testing beyond carbon source utilization to other metabolic processes. For example, nitrogen, phosphorus, and sulphur source tests could be developed, along with auxotrophy tests, antibiotic sensitivity tests, and tests for sensitivity to salt and pH extremes.

Biolog manufactures microplates with preselected sets of chemical substrates loaded and dried into each of the wells. Every well of the microplate also contains a complex, low-concentration nutrient medium along with the redox dye chemistry. Biolog's GN MicroPlate has a diverse set of 95 chemicals selected for their ability to discriminate species of Gram-negative bacteria. Because the panels are pre-loaded, all the user must do is inoculate the microplate with a cell suspension, a procedure that takes roughly one minute. After inoculation, the microplates are incubated at an appropriate temperature and read after 4 hours or 24 hours of incubation.

The microplates can be read most conveniently and accurately with a microplate reader and computer. The MicroLog software package developed by Biolog subtracts the background cell density from the negative control well and interprets all tests above a threshold as positive. Computerized matching of the metabolic fingerprint of the isolate to a large database of patterns in MicroLog identifies the microorganism under study. MicroLog can read all 95 test reactions and match the microplate pattern against known species (each with a family of patterns) to identify the microorganism within 15 seconds. An option allows users to add patterns to supplement the current database.

The 95-test format can generate 2^{95} (approximately 4×10^{28}) positive/negative patterns. If the tests are well-chosen, the approach can identify microbes with extremely fine resolution. Additional resolution can be obtained by following the colour changes in the wells kinetically instead of reading them as positive or negative after a fixed incubation period. Biolog's current database of reference patterns contains 310 species or groups of Gram-negative bacteria, 260 of which have been published in a recent manual. The database will be expanded to include more Gram-negative aerobes, Gram-positive aerobes, anaerobes and yeast.

Besides simply identifying unknown microbes, the testing process also yields useful and practical metabolic information. For example, it can aid in the development of growth media or selective enrichment media for the microorganism of interest. Data of this type are also being used by the US Department of Energy's Deep Subsurface Microbiology Program to characterize the degradative capabilities of indigenous soil bacteria in the hopes of using them for pollution clean-up.

The simple inoculation and the automated reading procedures of the technology allows a single microbiologist to perform efficiently several thousand metabolic tests in a single day. The computerization is also very useful for storing and analysing the vast amounts of data that can quickly be generated. The

Microbiology minutes

At next week's meeting of the American Society for Microbiology in New Orleans, Louisiana, products for growing, analysing and engineering microbes will be on display.

PROMEGA is announcing two new products: recombinant RNasin ribonuclease inhibitor and thioredoxin (*Reader Service No. 101*). Promega says its **RNase inhibitor** (\$68(US) for 2,500 units) protects the structure of RNA in a wide range of applications, increasing the size and yield of cDNA, maximizing the yield of full-length translation products, and enhancing RNA yield from transcription *in vitro* systems. The company's recombinant **thioredoxin** functions as a protein disulphide reductant as well as an isomerase, making it useful in the refolding of recombinant mammalian proteins produced in bacteria that do not generally produce disulphide-containing proteins. Prices range from \$90 (US) for 5 mg to \$360(US) for 25 mg.

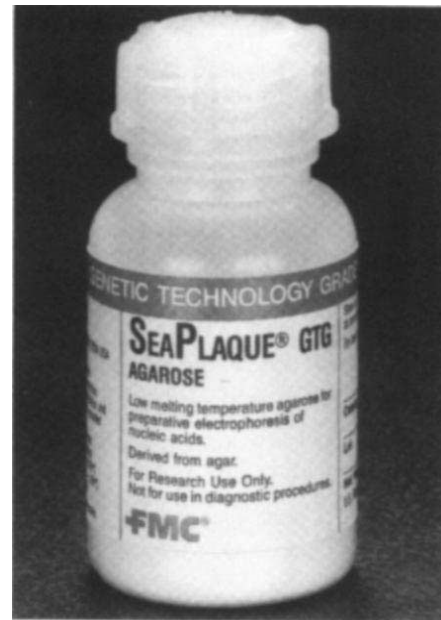
Media and buffers

A **blood agar base** formulated specifically for use with sheep blood is being introduced by Oxoid to meet the demand for a nutritious agar base that permits maximum recovery of organisms without interfering with haemolytic reactions (*Reader Service No. 102*). Oxoid has also extended its media range for detecting *Listeria monocytogenes* to include *Listeria*-selective enrichment broths. Because *Listeria* is transmitted to humans through meat, poultry, vegetables, seafood, and milk and other dairy products, the new media are useful in the food and dairy industries.

The new Seaplaque GTG agarose from FMC BioProducts was developed for the electrophoretic separation of DNA fragments larger than 1,000 base pairs (*Reader Service No. 103*). The company says the product is a low-temperature agarose ideally suited for the manipulation of nucleic acids directly in remelted agarose and that it saves time by eliminating the DNA recovery step. According to FMC BioProducts, Seaplaque GTG agarose ensures reliable results in ligation transformation, restriction digestion, nick translation and random priming. Seaplaque GTG costs \$86(US) for 25g.

speed, simplicity and efficiency that this technology provides accelerates the process of phenotypic testing by at least two orders of magnitude. It is a very practical approach for testing and identifying large numbers of diverse microbial isolates, and will facilitate large-scale surveys that were previously inconceivable. □

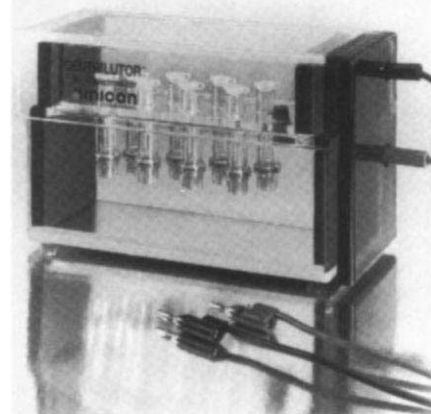
Barry R. Bochner is at Biolog, Inc., 3447



Seaplaque GTG agarose from FMC Bioproducts.

Electrophoresis etcetera

Amicon says its new Centricon-100 **micro-concentrator** offers a sample recovery rate of greater than 90 per cent and rapid desalting of DNA samples 2 ml or less in volume (*Reader Service No. 104*). Desalting or buffer exchange with simultaneous concentration can be performed in the



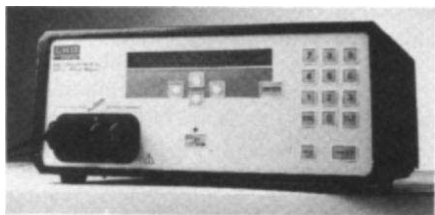
Works with the Centricon microconcentrators.

Investment Blvd., Suite 3, Hayward, California 94545, USA. For a copy of the Biolog reference manual, fill in reader service number 100. Biolog will be exhibiting in booth 101 at the American Society for Microbiology meeting.

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3. Bochner, B.R., US Patent No. 4,129,483 (1978).
4. Bochner, B.R., US Patent No. 4,235,964 (1980).

same unit. Amicon says the technique is less time-consuming and requires less sample handling than dialysis, gel filtration or chemical filtration. The Centricon units come with membranes with cutoffs ranging from 3,000 to 100,000 MW, for filtering out DNA fragments, proteins, viruses, yeasts and bacteria. The devices feature a concentration reservoir, a filtrate storage cup, retentate cup and cap. Up to nine Centricon units (\$3.20 (US) each) can be used with Amicon's \$495(US) Centrilitur micro-electroeluter to recover 1-25 µg of sample.

Pharmacia LKB's MultiDrive XL electrophoresis power supply can be used for most electrophoretic techniques (Reader Service No. 105). The easy-to-program power supply provides a constant current to 400 mA, constant voltage to 3500 V and constant power to 200 W. Nine methods, each containing nine steps, can be programmed and stored. The Multi-Drive XL offers four control modes, including time, volthour integra-



For electrophoresis up to 200 W.

tion, milliampere-hour integration or the derivative of current with respect to time. The unit's four high-voltage outlets allow four separations to run simultaneously. The MultiDrive XL is suited for use with isoelectric focusing, SDS-PAGE and pulsed-field gel electrophoresis. The power supply is only one of a host of new products featured in Pharmacia's 1989 Electrophoresis Catalogue.

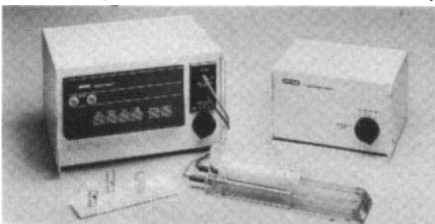
Beckman Instruments is expanding its range of reagents for pulsed-field gel electrophoresis to include **DNA standards** (Reader Service No. 106). New additions include DNA concatemers, and *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* whole chromosomes. Beckman prepares the standards with certified LMP agarose and tests them to determine correct migration and banding patterns. Testing procedures are performed using transverse alternating field electrophoresis. Beckman supplies each standard, at \$65(US) to \$125(US) per package, with a certificate of performance indicating precise molecular weight information.

Membrane miscellany

Millipore has recently introduced its Immobilon-N charge-modified, hydrophobic polyvinylidene difluoride **transfer membrane** for use in dot, slot, Southern and Northern blots, and multiple reprobe

analyses (Reader Service No. 107). Millipore says the high mechanical strength and chemical compatibility of the membrane contribute to its easy handling and superior results obtained with RNA and DNA. A package of 10 15 × 15-inch sheets is priced at \$85(US).

Bio-Rad's Gene Pulser **pulse generator** uses capacitor discharge to initiate electrical pulses for reversible permeabilization (electroporation) of cell membranes (Reader Service No. 108). Electroporation introduces nucleic acids, proteins and small molecules into cells, providing a reproducible method of transfection for mammalian cells, plant protoplasts and bacteria. The Gene Pulser is capable of producing a stable transfection efficiency



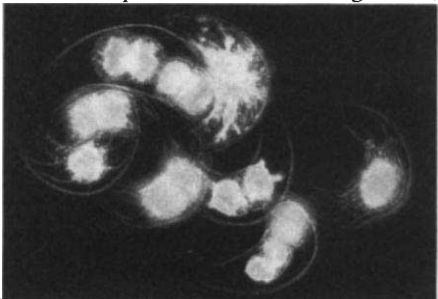
The Gene Pulser apparatus from Bio-Rad.

of greater than 10^{-3} transfectants per total cells and controlled exponential pulses with peak amplitudes from 50 to 2500 V, says Bio-Rad. Flexibility is provided by the wide range of field strengths, high current capability and large selection of capacitors.

Through the looking glass

Fluorescent objects, reflecting metal surfaces and low contrast specimens need no longer create problems for photomicrography. Wild Leitz says its Photoautomat microscope can cope with these difficulties by automatically calculating the correct degree of exposure (Reader Service No. 109). According to Wild Leitz, Photoautomat is the only worldwide system that determines exposures with 90 per cent of the available light but uses 100 per cent of the light in the film plane for the photograph itself. The system's memory can be digitally programmed with the work data specific to various tasks and different users.

Carl Zeiss is introducing a new **fluorescence microscope**, the Axioskop 20, which also provides results in brightfield,



Pyrocystis lunula under the Photoautomat.

darkfield and phase microscopy (Reader Service No. 110). The microscope's system-integrated design allows the acceptance of a fully integrated fluorescence system with full image brightness and large field of view. With its new 6-V, 20-W transmitted-light illuminator, the Axioskop 20 meets the needs of microbiologists and cell biologists. Zeiss says the system is equally suited for the observation of stained specimens, for haematological applications, and in darkfield

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The Zeiss Axioskop 20 microscope.

work with flagellates and phase contrast microscopy. The new infinity colour-corrected system objectives offer advantages in photomicrography and video documentation, because of their large numerical apertures and capability to produce high image contrast. The Axioskop 20 sells for \$8,216 (US).

The right environments

Labconco has a **controlled atmosphere glove box** designed for work in inert atmospheres or in a vacuum (*Reader Service No. 111*). The glove box is rated for a pressure drop not to exceed a 1-inch water



For controlled-atmosphere work.

gauge in two hours at a starting pressure of two inches. Labconco says the leak rate is less than 7 ml per minute at a two-inch water pressure, the maximum working pressure is five inches water gauge positive or 10 inches negative, and the transfer chamber may be evacuated to 50 μ m. The enclosure is constructed of one-piece moulded fibreglass-reinforced polyester with bonded double walls. The interior and exterior are corrosion fire and chemical resistant, and the glove box has no joints, cracks or crevices, allowing for easier wipedown and decontamination. The price of the glove box is \$6,645(US).

The 8400 series **ultra-low temperature freezers** introduced by Forma Scientific

maintain temperatures of as low as -85°C and feature Enviro-Scan, a micro-processor monitoring system (*Reader Service No. 112*). Enviro-Scan digitally displays actual chamber temperature and allows programming of desired high and low alarm limits. The monitoring system provides high/low alarm tests, reports residual battery power with a battery test feature, and monitors power line voltage. The freezer is maintained at operating temperature by two air-cooled hermetically sealed compressors. Freezer operation is guaranteed in an ambient temperature of over 90°F .

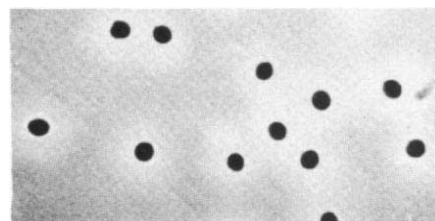
Shake, rattle and roll

Fisher Scientific now offers 26 models of **laboratory hotplates and stirrers** (*Reader Service No. 113*). The appliances are available with a choice of ceramic, porcelain or aluminum tops with baked enamel finish, in sizes ranging from 10×10 cm to 30×30 cm. The small aluminum top stirrer comes with an integral plate-support clamp for convenient mounting on latices and stands. Appliance bodies are sleek, stable, low-profile designs made of die-cast aluminum for durability and long life. Prices range from \$99(US) to \$308(US).

A new **shaking system** that accepts clips for all popular-sized shake, nephalo and Erlenmeyer flasks is available from Bellco Biotechnology (*Reader Service No. 114*). The system's continuous LED display of shaker speed and temperature feedback control loops, solid-state temperature sensor and optional multi-mode timer provide smooth, reproducible shaking at precise growth temperatures, says Bellco. The (\$1,575 (US)) shaker features safety enhancements such as fully closed shaker arms, built-in baffles, and a gable cover for reduced splashing at high speeds.

Separation and extraction

Micron Separations is announcing a new **polycarbonate filter** line that may eliminate the need for clearing membranes in microscopy analysis (*Reader Service No. 115*). The translucent filter allows for easier reading of the particles or cells, subsequently saving time associated with the clearing step when using mixed esters of cellulose. Ranging in size from 0.1–8.0 μ m, the filters are efficient for cellular, bacterial, protein and element analyses and for use with optical and electron microscopy, says the company. Depending



Polycarbonate filter from Micro Separations.

on size, the prices range from \$32(US) to \$126(US).

Designed totally to automate the proteinase K/phenol chloroform method of DNA extraction, the model 340A **nucleic acid extractor** from Applied Biosystems isolates high-molecular weight DNA with



The model 340A nucleic acid extractor.

minimal hands-on time (*Reader Service No. 116*). The extractor processes up to 24 samples per day. Samples are loaded directly onto the instrument and then undergo digestion, organic extraction and alcohol precipitation. The DNA is collected on a disposable filter and resuspended in a suitable buffer. DNA may be extracted simultaneously from fractionated or unfractionated peripheral blood, chorionic villi, cell cultures, bacteria and viruses. The routine isolation of high molecular weight DNA has applications in genetic disease research, cancer research, population studies, forensic science, paternity testing and pathology.

Shopping spree

Sigma Chemical Company is announcing the availability of its 1989 **molecular biology catalogue**, which lists a comprehensive range of biochemicals and products, including endonucleases, nucleic acid modifying enzymes, DNAs, reagents and equipment (*Reader Service No. 117*). New product areas include buffers for hybridization and electrophoresis and an expanded line of photographic equipment.

The 17th edition of the catalogue on **bacteria and phages** is now available from the American Type Culture Collection (*Reader Service No. 118*). The 552-page reference contains over 12,000 bacteria and phage strains as well as media formulations for growing specific cultures. A cross-referenced index is included for matching the alphabetical and numerical strain designations used by various laboratories with the corresponding ATCC culture numbers. □

These notes are compiled by Cary Prince from information provided by the manufacturers. To obtain additional information about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.